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CHOLERA CARRIERS IN RELATION TO CHOLERA CONTROL [1]

By E. L. MUNSON

(*Major, Medical Corps, United States Army. Advisor to the Bureau of Health*)

In the recent cholera outbreak in Manila and in Bilibid Prison a very high percentage of carriers in persons not cholera suspects was early discovered. The number of both carriers and cases was showing a steady increase, and there were a number of recurrent cases developing in groups found to have a high carrier index. It was then decided to depart from previous ideas, and to regard actual cases merely as symptomatic of a wider spread and more dangerous concealed infection.

The dangerous nature of frank cases of cholera with diarrhœa, vomiting, and collapse is well recognized by the people, and such cases would be avoided, reported, isolated, and followed by disinfection of their environment. But the carriers were unsuspected sources of infection who were scattering it broadcast through the public latrines, handling and preparing food and drink for public consumption, and admitted as welcome guests into homes and social entertainments. To avoid a danger, it must first be known where it exists.

Special effort, therefore, was made to seek out and isolate the carriers, who were presumably the chief factors in spreading the infection. It was recognized that the task would be one of too great magnitude to include all the inhabitants of the entire city, nor was this thought necessary in practice; so, after a few special surveys to determine the degree of prevalence of infection, the work of carrier detection was limited to contacts with cholera cases, to persons living in a vicinity in which several cases had occurred, and to persons engaged in the handling of food and drink in hotels, restaurants, bottled-

[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

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drink factories, clubs, tiendas, and ice-cream factories, by which classes infection was particularly liable to be transmitted. Some surprising results were obtained with the last class, and numerous carriers were found and removed from duties in which they might have caused—and doubtless did cause—much damage.

To meet the needs of the work, the Governor-General authorized additional expenditures which doubled the capacity of the Bureau of Science with regard to cholera diagnosis by bacteriological methods. However, after funds were authorized, it took several weeks to secure and install the necessary additional equipment and to obtain and train the additional personnel. Finally, bacteriological examinations were being made at the rate of nearly 2,000 per day, due to improved organization and technical efficiency of the new employees of the Bureau of Science.

With the increased isolation of carriers thus made possible, the cases began to fall. The epidemic appeared to be undermined rapidly at its source. At one time there were 195 carriers in detention. The practical importance of having removed such a number of concealed foci from the community at one time and put them where they could be of no danger in spreading the infection thus harbored does not need to be emphasized. During the outbreak, 529 cholera carriers were found through the health stations and 128 more occurred among the convicts of Bilibid Prison. A total of 657 such carriers was thus found and isolated within the corporate limits of Manila up to the middle of November.

The following is the report of stool specimens taken from persons not cholera suspects and forwarded to the Bureau of Science for examination during the months of July, August, September, and October, 1914, as taken by the several health stations in the city of Manila:

TABLE I.—*Stool specimens examined for cholera at the Bureau of Science. July–October, 1914.*

Source of specimen.	July.			August.			September.		
	Total.	Posi-tive.	Nega-tive.	Total.	Posi-tive.	Nega-tive.	Total.	Posi-tive.	Nega-tive.
Health station—									
J	238	27	211	1,327	45	1,282	3,539	69	3,470
A	830	31	799	4,851	118	4,733	5,467	73	5,394
C	304	13	291	647	27	620	1,796	26	1,770
I	95	5	90	736	18	718	1,414	21	1,393
L	77	1	76	80	....	80	840	16	824
Total	1,544	77	1,467	7,641	208	7,433	13,056	205	12,851

TABLE I.—*Stool specimens examined for cholera at the Bureau of Science, July–October, 1914*—Continued.

Source of specimen.	October.			For the four months.		
	Total.	Posi-tive.	Nega-tive.	Nega-tive.	Posi-tive.	Total.
Health station—						
J	1,153	6	1,147	6,257	147	6,304
A	2,800	22	2,778	13,948	244	14,192
C	1,489	4	1,485	4,236	70	4,166
I	513	4	509	2,758	48	2,806
L	1,192	--------	1,192	2,189	17	2,206
Total	7,147	36	7,111	29,448	526	29,974

It is interesting to note the way in which the cholera carriers have fallen off. In October, including Bilibid Prison, whose examinations are not included in the above statistical table for health stations, a total of 80 carriers was found. Of these 80, there were 57 found in the first half of the month and 23 in the last half of the month. Of the 23 carriers found in this last half-month, only 8 occurred in the last week of October. In the first week of November, only 3 carriers were found. In the first twelve days of November, as a result of approximately 20,000 examinations made during that period, only 3 carriers were found. The last carrier was found on November 4, since which date up to the present writing some 16,000 examinations have been made.

The foregoing statistical table shows that for the entire outbreak, up to November 1, when it was practically over, of nearly 30,000 persons not cholera suspects but systematically examined from health stations for purposes of investigation, almost exactly 1.75 per cent of the population of Manila examined were found to be harboring the cholera infection. But such general proportion was much exceeded in some instances. Station A in August showed 118 positives in 4,851 instances taken at random, or 2.4 per cent. One series of 179 dead bodies showed 3.6 per cent positive; and certain smaller groups among living persons showed even higher percentages of infection than the foregoing. The Bilibid Prison outbreak, which is not discussed in this paper, has had approximately 5 per cent of the prisoners found to be cholera carriers. Fortunately for the work of eradication, only a part of the infections above mentioned for large groups existed at any one time.

In some instances, cholera infection was found no more prevalent in cholera contacts than in those not known to have had any relation to cholera cases. Thus at Station L for the month

of September 149 cholera "contacts" showed 3 positive, or 2 per cent, while of 691 specimens taken systematically from the inhabitants of city blocks, 13, or almost exactly 2 per cent, were positive. The same applies to Station J for September, with 57 positives out of 2,970 systematic examinations of nonsuspects, and 12 positives out of 541 contacts.

The above statistical table also well shows the invasion of Paco district by the infection, due to being conveyed by the floods of September. Prior to the floods it had been practically free from cases—afterwards, both cases and carriers increased in almost parallel ratio.

The vast amount of assistance given the health work by the Bureau of Science is shown by Table II.

TABLE II.—*Number of examinations for cholera made at the Bureau of Science and number found positive for four months in 1914.*

Month.	Examinations for cholera.	Specimens found positive.
July	3,332	137
August	9,994	652
September	24,402	989
October	32,824	191
Total	70,552	1,969

From November 1 to November 10 approximately 20,000 bacteriological examinations were made. Cases and carriers have both now fallen almost to the vanishing point, and the examination for cholera of apparently healthy persons will shortly be discontinued. However, when so discontinued, well over 100,000 such examinations will have been made.

Of the above gross figures, a considerable proportion of the positive findings represent subsequent examinations of cases previously found positive which were being examined to determine the time when they had cleared up as carriers and could be discharged from isolation.

It will be apparent from the above that the work of detecting cholera carriers presented grave difficulties of administration by reason of its magnitude. There were also social and political difficulties which had to be overcome before it was possible to undertake the purely scientific and administrative work. The work meant invasion of the accepted rights of the home and of the individual on a scale perhaps unprecedented for any community. The collection of the fæcal specimens necessary might fairly be regarded as repulsive to modesty. Add to this the facts that the search was made among persons apparently healthy to

themselves and others who could scarcely fall even within the class of suspects, and that those found positive were subjected to all the inconveniences of isolation, separation from family, loss of earning capacity, etc., and it is apparent that the work at the outset had to be expanded with caution and only as the original opposition could be removed and public opinion created in favor of it as an unpleasant but necessary measure. This was brought about rapidly in various ways, and by the middle of September the full support of every newspaper in Manila had been secured, together with that of practically all persons of prominence and of the intelligent classes. It is a credit to the people that they accepted the work as a necessity, for without their coöperation the work could probably not have been carried out. By the unprecedented floods of September the greater part of Manila was put several feet under water for some days, sewers were back pressured, most of the public water closets upon which the great majority of the population of Manila depend were submerged, and the poorer people were forced to drink the foul flood water which could be boiled with difficulty from lack of fuel. There was also shortage of food, constant wetting and chilling from unceasing rain and flood, and crowding together of people driven from their homes by rising waters and carrying their infection into new places. These conditions and the existence of a widespread cholera infection in carriers apparently favored the development of one of the most destructive epidemics of cholera that ever occurred in Manila. That it did not so occur probably can be attributed largely to the campaign against carriers which shortly after began to be pushed more energetically, through better organization and allaying popular opposition. The flood began on September 3. Within a week, the number of cases and carriers rose rapidly. In the health districts of Manila and in Bilibid Prison together there were 226 carriers found and isolated for the period September 8 to September 30. On one day, September 14, there were 41 carriers found, and on September 15 there were 52 carriers found.

It is evident from the above that in effectively combating a cholera infection the use of laboratory facilities in the making of bacteriological diagnosis on a large scale is absolutely essential. Without such assistance as the Bureau of Science has given, the results accomplished would not have been possible.

The outbreak in Manila was unquestionably spread chiefly by personal contact. Cases were isolated so promptly as to do little harm. Lack of the use of toilet paper, certain habits

in the use of the toilet, infected fingers, and eating with the hands food taken from a common dish were the channels through which the infection chiefly passed from the carrier to another person.   Public water supplies and articles of food could be eliminated as channels of infection, and flies played an entirely insignificant part in its spread.

A considerable proportion of the positive specimens taken from dead bodies were from cases in which the cause of death was reported as enteritis, diarrhœa, dysentery, infantile beri-beri, and pulmonary tuberculosis.   The coöperation given by all the physicians of Manila in the detection and isolation of cholera was so genuine that it is not believed that there was any effort to conceal cases under other diagnoses.   Errors in diagnosis were made in good faith.   Also, it was quite possible for persons to die of one disease and still be carriers of another, or harbor an infection which had not yet time to develop. The proportion of cases of pulmonary tuberculosis found positive for cholera on examination of the fæces is notable.   Here the tubercular lesions of the intestines in advanced cases apparently played a considerable part in rendering the alimentary tract a more favorable environment for development of cholera germs.   So also with the other intestinal diseases mentioned. Apparently almost any intestinal disorder or interference with intestinal digestive function materially predisposes to development of cholera infection if the latter gain access to the alimentary tract.

At the outset of the campaign for the detection of cholera carriers, the accuracy of the microscopic diagnosis made by the Bureau of Science was called into question by various persons, who objected that in their opinion true cholera germs could not be present as reported, as no carrier was developing the disease.   To this objection reply was ordinarily made that the fact that the persons harboring the cholera germs were carriers rather than cases was because they possessed such temporary powers of resistance to the germs as to be able to prevent the development of the disease.   However, it was believed that cases of cholera might very well occur in carriers as a result of either decrease in vital resistance of the host or increase in virulence of the strain of germ being harbored; and very shortly a considerable number of cases occurred to prove this to be a fact. For example, in the search for carriers in Bilibid Prison, convicts 8617, 12765, and 30351 were reported as positive carriers on September 10 and 11.   They were isolated and examined bacteriologically every other day, being found continuously pos-

itive for cholera. Convict 8617 developed true cholera on September 27, after being a carrier for seventeen days; convict 12765 developed true cholera on September 27, after being a carrier for sixteen days; and convict 30351 developed cholera on September 29, after being a carrier for eighteen days, and the disease was of a type sufficiently severe to cause death in eight hours.

Several instances have occurred where persons who had given specimens became suspicious of possible findings and absented themselves from their usual abodes, so that when later determined by the Bureau of Science to be positive they could not be found and segregated—only to be taken up later as true cholera cases in some other portions of the city. And many cases have occurred in which the disease has developed within the usual period of incubation, such as convict 8486, who was found to be a carrier on September 16 and developed active symptoms of the disease on September 20.

What may be the cause of development of the symptoms of cholera in carriers can only be surmised with our present knowledge of the disease. But it is worthy of note that in the Bilibid cases at least no causes which might operate to produce a general lowering of vital resistance were apparent. They were isolated, at rest, well fed, and under every hygienic advantage.

A number of cases of intermittent carriers have been found, and it would probably be shown that these are not rare if reexamination of all carriers for a considerable period could be carried out. Thus, Mamerto Juanico was found to be a carrier on September 16, but was released from quarantine on October 4 after four negative findings, approximately at two-day intervals. But on October 26 this case was again found positive and so continues at the present time. Here is a case which has been an intermittent source of danger for over seven weeks. If it can be a carrier for seven weeks, why not for fourteen? If the germ can be harbored any such length of time, what is the limit of its viability in the intestine? Clearly such cases tremendously increase the difficulty of cholera control.

Take the case of Alejo de la Cruz, who was found positive on September 27 and 29 and then was negative for 4 findings and released from quarantine on October 14; but who was again found positive on October 17, developed choleraic symptoms on the same date, and was sent to San Lazaro where he remained until October 27. Was this last manifestation a suddenly increased virulence of a previously existing but scanty infection, or was it a sudden lowering of vital resistance, or

did the man acquire a new and more virulent infection which caused him to sicken?

Apparently healthy persons in quarantined barracks in Bilibid Prison, from which carriers were being carefully sought out and removed by means of bacteriological examinations made every other day, have produced cases in from two- to three-day up to twelve- and thirteen-day intervals. This is suggestive of persons being able to harbor an infection which is undetectable by present methods, or else of a much longer incubation period than has previously been accepted.

One instance is reported of a man who was treated as a case of cholera at San Lazaro Hospital in 1913 and this year was found to be a cholera carrier and sent there for detention. The question at once presents itself as to whether or not he might perhaps have been a host for the cholera germ since his previous sickness, and thus be representative of a class whose existence in these Islands may reasonably be inferred from the practically annual recurrences of this disease and by which the gaps between one outbreak and another may very possibly be bridged over.

Treatment to free the intestines of cholera carriers of cholera vibrios seems to have been of little value. It will be discussed in another paper at this meeting. It is worthy of note that 4 cases of cholera, 1 case fatal, occurred in carriers who had for some time been receiving salol in 0.6 gram doses twice daily in the effort to hasten the disappearance of cholera germs through the use of intestinal antiseptics.

As to the duration of the period in which the average cholera carrier spontaneously cleared up, there seemed to be some variation. Average days of detention were reported from San Lazaro as follows:

TABLE III.—*Average time of detention of cholera carriers at San Lazaro.*

Month.	Males.	Females.
	Days.	Days.
July	7	8
August	8	6
September	10	11

However, very likely some of these cases which were reported as cleared up were intermittent carriers and again became temporary disseminators of disease germs, and thus the average period of infectivity as given above is probably too short.

Experience would seem to indicate that in an outbreak of

cholera presenting a high case mortality, the proportion of persons who are carriers without presenting symptoms of the disease will be relatively small. Possibly this may be due to the fact that in such an epidemic the strain of germ concerned is so virulent that if introduced into the system the average power of vital resistance is insufficient to check the invader and the host promptly sickens and usually dies. Conversely, where the case mortality is light, the lack of virulence in the germ will probably permit it to be harbored in many cases without the production of symptoms, and a considerable percentage of carriers may be expected.

The recent outbreaks in the provinces and Manila have presented most clearly these two distinct types of infection: the provincial case mortality has been nearly twice that of Manila and relatively very few cholera carriers have been found.

But we must bear in mind the possibility that an apparently mild strain of cholera germ, under conditions of environment as yet not fully understood by us, may acquire a high degree of virulence and change the type of disease from one of a relatively benign character to one of a most fatal type. This adds to the necessity of seeking out and removing the concealed sources of infection found in cholera carriers.

One of the most apparent lessons to be learned from these recent experiences relates to the possible period of latent infection in cholera and its bearing on the period of incubation and quarantine heretofore accepted for health work. It is undoubtedly true that the five-day period usually accepted for incubation and quarantine ordinarily will suffice for the control of infection in the majority of cases; but it is equally true that such a period does not hold good in a very considerable number of instances, which sheds much light on cholera situations not otherwise readily explainable. For example, convict 30351, who died of cholera, might have traveled halfway around the world, scattering his infection broadcast during his eighteen-day period as a carrier, and died of true cholera in a place many thousands of miles from any other source of infection. There is a warning in such cases that health officers all over the world would do well to heed.

In conclusion, in such outbreaks as that recently in Manila, the carriers would seem to be not only the most numerous but the most insidious and dangerous sources of infection. The prompt eradication of a general cholera infection, therefore, includes the detection and isolation of carriers as a scientific prerequisite.

OBSERVATIONS CONCERNING CHOLERA CARRIERS [1]

By Otto Schöbl

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

The condition frequently existing in persons termed by English authors "carriers," "distributors," or "porters," being common to all intestinal bacterial infections, is found in Asiatic cholera, and in the case of chronic carriers is without doubt due to the infection of the gall passages by cholera vibrios.

The portal of entry as well as the principal field of cholera infection in man is the intestinal tract, and it is quite natural that we look on the intestinal discharge as the main source of supply of the infectious material. Nevertheless we are led by experience in typhoid fever, a disease which has much in common with cholera, to search for other less commonly infected excretions by means of which cholera vibrios may be discharged from the human body. The urine and the vomit of patients may be mentioned as examples.

The first question of practical interest with regard to cholera carriers is: "How long is a cholera convalescent infective?" The following quotation from Greig [2] answers the question: "It is impossible from an ordinary medical examination to say 'whether or not a patient is infective.'" The bacteriological diagnosis, which consists of isolating and identifying the specific vibrio, requires a fairly well-equipped laboratory and an experienced personnel. These are not always available; therefore the data on the vitality of cholera vibrios in the human body may be of practical value under these circumstances.

The results of the examinations of about 80 cholera patients and carriers gave the following figures:

TABLE I.—*Outbreak of cholera in Manila, 1913–1914.*

Stools positive:	Patients.
For from 2 to 7 days	43
For from 7 to 14 days	22
For from 14 to 21 days	6
For from 21 to 28 days	5
For 48 days	1

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Pfeiffer [2] mentions only 2 cases of from forty-eight to forty-nine days' duration; Stühlern-Zeidler,[3] 1 case of ninety days; Jakowleff [4] gives 1 case of fifty-six days; and Creel,[5] 1 case of fifty-eight days' duration. Our record case was positive for forty-eight days with several intermissions. The case was in the care of Dr. C. S. Butler, of the United States Navy.

According to the clinical report, which I obtained through the kindness of Doctor Butler, the patient became sick on October 5. The fæces were still positive on November 23. Three negative examinations three days apart followed.

It is interesting to note that the chronic carriers of the Russian authors showed intermittent diarrhœa after recovering from the acute attack of cholera. It also was noticed that chronic carriers exhibited clinical signs of cholecystitis; that is, icterus and tenderness in the region of the gall bladder.

In a recent páper Greig [6] tabulated the results of 271 bacteriological examinations of gall bladders taken from deceased cholera cases, and emphasized the significance of the already known fact that cholera vibrios frequently are found in the bile passages of cholera patients and convalescents. His is the largest series of examinations on record. Among the 271 examinations the cholera vibrio was found eighty times, and 12 of the 80 gall bladders which harbored cholera vibrios showed pathological changes.

Kulescha [7] studied the pathology of bile passages during the outbreak of cholera in St. Petersburg in 1908–1909. He found in the literature the first report of a necrotic cholecystitis in a case of cholera by Pirogoff (1848) and an analogous case by Netschaeff (1892). Mentioning the numerous authors who contributed to the knowledge of the subject, he quotes the results of examinations made by M. J. Girode as of particular interest. Of 28 cases examined, 14 contained vibrios. There was one case of marked cholangitis. Savtschenko found cholecystitis twice among 30 cholera autopsies. Kulescha found, among 430 autopsies performed on cholera cadavers, cholecystitis in 10 per cent. The majority were in the first or second week of the disease. Cholera vibrios were found in 46 per cent of gall bladder examinations in 1908 (109 cases examined) and in 76

[3] Cited from Jansen, *Klin. Jahrb,* (1910).
[4] Cited from Kulescha, *Klin. Jahrb.* (1910).
[5] *Journ. Am. Med. Assoc.* (1912), 187.
[6] See footnote 2.
[7] See footnote 4.

per cent of cases in 1909 (50 examinations). His is the unique case of a patient who took sick with cholera in November, 1908, and became a carrier (fæces positive for cholera vibrios for fifty-seven days). Death occurred in September, 1909. Cholera vibrios were found in the bile passages, but not in the fæces.

The close relation between the infection of the gall bladder and the condition in convalescents known as cholera carriers was early recognized. Nevertheless the emphasis of the fact and its importance in regard to the dissemination of the disease is justified, because assertions to the contrary are to be found in the literature as evident from the statement attributed to Roger by Greig:[8]

> The absence of the infection of the gall bladder and bile ducts by the comma bacillus places the disease in quite a different position from that of typhoid fever in this respect.

Considering the lengthy period of infectiveness as found in certain instances of cholera carriers and the periodical reoccurrence of cholera vibrios in the stools of convalescents, theoretically it would be difficult to believe that the cholera vibrio would live for such a length of time free in the intestinal tract where the competition with the normal inhabitants of the intestine and other factors render the conditions unfavorable to its vitality.

The tidal occurrence of the cholera vibrio in the stools of convalescents who become carriers seems to indicate a focus connected with the alimentary canal, where the vibrios multiply and are being discharged into the digestive tract. At times and under certain conditions they appear in the excreted fæces in numbers large enough to be detected by the usual methods.

As to the genesis of the infection of the bladder and the bile ducts two ways come under consideration. Does the invasion of the bile passages take place directly from the small intestine or is the infection of hæmatogenous origin? The facts that the bile passages show marked pathological changes while the liver tissue proper exhibits, as a rule, only signs of toxic effect, the high percentage of infected gall bladders, and the rarely encountered evidence of a bacteremic stage of cholera infection speak in favor of the first-mentioned mode of infection.

It was found that bile is not only a fairly good medium for the growth of the cholera vibrio, but also that it inhibits the growth of many other intestinal bacteria. As a matter of fact,

[8] See footnote 2.

it was recommended as enrichment medium.   During the acute attack of cholera the proximal part of the small intestine usually contains cholera vibrios in pure culture, and frequently cholera vibrios are found in the stomach contents if vomiting sets in. Two out of three vomits collected from known cholera cases examined by me contained numerous cholera vibrios.

It is evident that once the lively motile cholera vibrios reach the gall bladder they grow practically without competition.

Kolle and Schürmann[9] state that the numerous examinations of cholera cadavers made in India showed that the cholera vibrios are restricted to the intestines while the internal organs are free from vibrios.   Their statement is based on the findings which were published in extenso in the official report of Professor Gaffky.

Greig[10] believes that the infection of the bile passages is of hæmatogenous origin.   He found cholera vibrios in a focus in the lungs.

Kulescha[11] admits that under certain conditions cholera vibrios invade the gall bladder through the bile passages, but he upholds the theory that the vibrios reach the gall bladder through the blood stream on the following ground: he found necrotic foci (emboli) in the liver of cholera cadavers and succeeded in isolating the cholera vibrios therefrom.   As a support of the theory of the hæmatogenous origin of the infection of the gall passages by cholera vibrios, this author quotes the findings of Sewastjaneff, who found 5 cases of vibrionuria, one of them being of four days' duration, and also the case of Liefschütz-Jakowleff who isolated the cholera vibrio from a stillborn child whose mother suffered with cholera.   Kulescha made *numerous examinations of urine in cholera patients under strict aseptic precautions and failed to find the cholera vibrio.*   In a preliminary note Greig[12] reports 8 positive findings of cholera vibrios in the urine (55 examinations).   Several cases analogous to that of Liefschütz-Jakowleff came under my observation during the outbreak.   The results of these examinations are summarized in Table II.

[9] Cited from Kolle und Wassermann, Handbuch der pathogenen Microorganismen.   Gustav Fischer, Jena.

[10] See footnote 2.

[11] See footnote 4.

[12] See footnote 2.

TABLE II.—*Examination of embryos from cholera mothers for the presence of cholera vibrios.*

[The cause of death in the mothers was Asiatic cholera.]

Case.	Waters.	Placental blood.	Heart.	Intestine.	Spleen.
1. Full-grown child _____	0	0	0	—	—
2. Full-grown child _____	0	0	0	—	—
3. Almost full grown _____	a +	—	—	-	0
4. Fœtus 24 centimeters_____	—	—	—	—	0
5. Almost full grown _____	—	—	—	—	b —

ª In case 3 the sac was found perforated, which explains the presence of cholera vibrios in the waters.

ᵇ Bile.

Of the 39 gall bladders examined for the presence of the cholera vibrio, 3 showed macroscopic lesions. In 2 instances (1 and 25) hydrops cystitis fellea was found; that is, distended gall bladder containing mucous bile of light-amber color and flaky sediment. When stirred, the bile assumed a milky appearance.

One gall bladder was rather small; the wall was evidently thickened, and the contents were of a rather dark color. Upon microscopical examination the epithelium was found desquamated, the blood vessels distended, and the mucous membrane showed a high degree of round-cell infiltration. Blood corpuscles were found free in the lumen of the gall bladder. The cystic duct showed like changes, but the epithelium was not desquamated altogether. Pure cultures of the cholera vibrio were obtained from all three specimens.

TABLE III.—*Showing the results of bacteriological examinations of gall bladders for the presence of cholera vibrios.*

No.	Patient.	Date of examination.	Time between death and autopsy.	Cholera vibrios.	
				Gall bladder.	Intestine.
		1913.	*Hours.*		
1	A. V	Sept. 22	16	a +	+
2	R. Q	Oct. 4	15	—	+
3	F. Unku	Oct. 6	6	—	+
4	T. D	Oct. 5	16	+	+
5	S. Q	Oct. 9	4	—	+
6	C. E	...do	14	—	+
7	F. Jap	...do	17	+	+
8	S. Jap	Oct. 11	20	—	+
9	De L. R	...do	5	+	+
10	M. Jap	...do	3	+	+
11	A	Oct. 13	(?)	—	..
12	A. P	Oct. 14	4	—	+
13	J. C	...do	11		+
14	C	Oct. 15	8		+
15	N. T	Oct. 14	16	—	+
16	J. B	...do	3.5	..	+
17	V. H	Oct. 13	6	—	+
18	G. P	...do	11	—	—
19	E. S	Oct. 16	7	+	+
20	F. B	Oct. 17	15	—	+
21	N. S	...do	4	—	+
22	M. L	Oct. 20	1	—	+
23	N. O	...do	2	—	+
24	R. S. A	...do	4	+	+
25	F. S	Oct. 23	12	a +	+
26	S. G	Nov. 26	(?)	b +	+
		1914.			
27	A. J	July 11	20	+	+
28	E. de G	July 14	0	+	+
29	M. L	June 14	0	+	+
30	M. Sta. M	July 16	0	—	+
31	V. (?)	July 21	0	+	+
32	J. Z	July 24	0	—	+
33	A. C	July 28	0	—	+
34	M. R	...do	0	—	+
35	A. B	July 29	0	—	+
36	A. R	July 31	0	+	+
37	C. A	Aug. 31	0		+
38	A. Z	Aug. 7	0	—	
39	I. P	...do	0	—	+

a Hydrops.   b Cholecystitis.

It will be seen from Table III that in the 39 gall-bladder examinations the cholera vibrio was found seventeen times. The period of time between death and autopsy as far as obtainable is indicated in the table.

TABLE IV.—*Showing the results of examinations of urine of cholera patients for the presence of the cholera vibrio.*

Patient.	Date of examination.	Cholera vibrio in—	
		Urine.	Fæces.
	1913.		
D. C	Sept. 30	—	+
	Oct. 1	—	+
	Oct. 8	—	+
	Oct. 11	—	+
	Oct. 17	—	+
B	Sept. 30	--	+
	Oct. 1	—	+
F	---do ----	—	+
	Oct. 8	—	+
T	Oct. 11	--	+
	Oct. 24	--	—
P	Oct. 11	--	+
M	Oct. 17	--	+
D	---do ----	—	+
	Oct. 23	—	+
	Oct. 24	—	+
L	Oct. 22	—	+
B. L	---do ----	--	+
T. M	---do ----	—	+
Mon	---do ----	—	+
	Oct. 24	—	+
T. Y	Oct. 22	--	+
	Oct. 24	—	+
Monte	Oct. 23	--	+
	Oct. 24	--	+
	Oct. 25	--	+
Cast	Oct. 23	—	—
	Oct. 24	—	—
Quin	---do ----	—	+
M. M	---do ----	—	+
W. L	---do ----	—	+
Lap	Nov. 17	—	—
Jim	---do ----	—	+
Tomago Y	---do ----	--	--
M. S	---do ----	—	+
	1914.		
P. B	July 22	—	+
	July 24	--	—
L. L	July 22	—	—
	July 24	—	—
T. M	---do ----	--	+
V. M	---do ----	—	+
S. M	---do ----	--	--
D. F	Aug. 1	—	—

All of the 41 examinations of 27 patients and convalescents, whose stools contained cholera vibrios at the time of examination or some time previous, were negative. It tends to show that vibrionuria is not a common occurrence in cholera asiatica.

THE DEVELOPMENT OF THE EGGS OF ASCARIS LUMBRICOIDES [1]

By Lawrence D. Wharton

(*From the Zoölogical Laboratory, College of Liberal Arts,
University of the Philippines*)

The great frequency with which *Ascaris lumbricoides* is found in the Philippine Islands and the unusual number of cases which have been reported recently in which the worms have been the direct cause of death or important secondary causes, through migration into the liver or pancreas, makes the study of their development of considerable importance to us, for it is only through a knowledge of the development, whereby we may obtain knowledge of the means of infection, that we may expect to decrease or eradicate these worms in the Philippine Islands. It was with this idea in mind that the study of this interesting form was undertaken; and, although my work so far has resulted in nothing of great moment, I think that some of the facts which have been obtained will be of interest.

In my experiments I have depended almost entirely upon eggs laid in the laboratory by living worms which have been obtained from the morgue through the kindness of Dr. B. C. Crowell. It was found that if ascarids taken from the intestine are placed in Kronecker's salt solution (normal salt solution to which 0.06 gram of sodium hydroxide per liter is added) they will remain alive and active for from six to twelve days and the females will generally lay a large number of eggs.

To obtain the eggs for experiment, separate adult females were placed in glass dishes of the solution and each worm was removed into a fresh dish as soon as any eggs were laid. The majority of the worms laid eggs only two or three times, but some laid as many as eight times before dying. The female worms were always kept in the light during the daytime and the eggs were generally, although not always, laid at night.

The eggs laid in Kronecker's solution differ in no way from those which are found in fæces. The commonest form is the typical oval egg consisting of a rounded mass of protoplasm

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[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

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surrounded by a shell composed of 2 thin tough layers of chitin
with a thick outer albuminous layer raised into blunt knobs or
mammillations. In addition to this form, the various atypical
forms which are sometimes encountered in fæces were also
found in the laboratory. The smooth eggs without an outer
albuminous layer of shell, which have sometimes been considered
to be the eggs of another species of *Ascaris,* are the commonest
atypical form. They are always laid in the laboratory after
the worm has been kept in Kronecker's solution for some days,
and are undoubtedly due to the failure of the glands of the
uterus to function on account of lack of nourishment. Among
the eggs obtained from 56 female ascarids, the first laying
consisted of typical mammillated eggs in every case except one
in which eggs were laid. In those cases where the worm con-
tinued to lay eggs the albuminous layer became thinner and
in the last layings obtained disappeared entirely.

THE DEVELOPMENT OF THE EGGS

The time required for development of the eggs under natural
conditions is much shorter than in countries farther north.
Most European and American authors state that the time of
development is from several weeks to six or eight months.
During March, April, and May eggs developed in from ten to
fourteen days when kept in the laboratory at the ordinary
temperature.[2] The eggs developed more rapidly and more reg-
ularly on the surface of moist earth or on earth covered with
a thin layer of water than in any other medium. Development
was also rapid in tap water, pond water, and Kronecker's solu-
tion when the eggs were spread out in flat dishes. In distilled
water the development was very irregular, most of the eggs
dying before the embryos were formed. In solutions containing
0.5 per cent of hydrochloric acid, 0.5 per cent of carbolic acid,
or 3 per cent of acetic acid, the eggs commenced their develop-
ment very quickly and developed at first very rapidly, but after
a few days development ceased entirely and the embryos died.
Since performing these experiments, I have read of some work
on the development of the eggs of the pig and calf ascarids
in which solutions of various acids of the strength of 1 part
in 1,000 were used, with the result that the development was
very much accelerated and continued until the embryos were

[2] The most rapid development I have found reported is by Leuckart, who
developed the eggs in fourteen days by keeping them in an incubator at
30° C.

completely developed. It will be interesting to determine if the eggs of these forms are more resistant to acids or if a difference of 3 parts in 1,000, of, say, hydrochloric acid, is sufficient to stop the development. In weak solutions of formalin and of potassium permanganate the embryos also began to develop, but died in a few days. The imperviousness of the shell is a source of constant surprise. On two occasions the uterus of a female containing eggs was left in 3 per cent nitric acid over night to fix it for sectioning and later it was found that the eggs had divided into 2 cells.

A small amount of moisture is a necessary requirement for the development of the eggs, although drying does not kill them. Eggs dry out so quickly on glass plates that they do not begin to develop. After fourteen days and again after twenty-one days some of them were placed in water; they began to develop, the first in twenty-four hours and the others in less than forty-eight hours. Some eggs were allowed to dry on earth. As the earth dried out very slowly most of the eggs began to develop. As soon as the eggs became dry the development ceased, but began again when the earth was moistened.

The eggs will not develop without oxygen, although the amount needed seems to be very small. If one or two eggs in a drop of tap water are sealed in a hanging-drop slide they develop as well as when exposed to the air. However, some eggs were introduced into water which had been boiled and were covered with a layer of oil to prevent the entrance of air; none of them had begun to develop after seven days, but they did not die as they began to develop as soon as they were placed in fresh water. If a large mass of eggs is put into a deep narrow dish with a small surface they will not begin to develop. I have kept them in this way for a month without results; they began their development as usual as soon as they were put into fresh water in shallow dishes.

Temperature undoubtedly has more influence on the development of the eggs than any one other factor. The most favorable temperature for development is about 30° C. At 37° development will begin, but all of the eggs die either in the 4- or the 8-cell stage. If eggs which have partially developed are placed in an incubator at this temperature development immediately ceases and they die. Exposure of the eggs to a temperature above 37° rapidly causes death. When eggs are spread on glass and dipped into water at a temperature of 70° for five seconds none of them develop. Eggs which contained well-developed embryos were placed in tap water at 70° and

allowed to cool.   They were all killed.   This point undoubtedly is of considerable practical importance in preventing the spread of infection.   Many fruits and vegetables which are commonly eaten raw can be dipped into water at this temperature without being injured.

Moderately low temperatures simply retard the development without killing the eggs.   Eggs kept for twenty-four days at a temperature between 5° and 12° showed no traces of development.   At the end of that time they were placed in the laboratory at ordinary temperature and developed into adult embryos in fourteen days in the same medium in which they were kept throughout the experiment.

THE HATCHING OF EMBRYOS

The embryo, when ready for hatching, is a small worm with a blunt anterior and a pointed posterior end.   It is coiled in the shell and moves almost constantly as long as it is alive. It is from 0.12 to 0.20 millimeter long and from 0.014 to 0.02 millimeter in diameter.   In tap water and in salt solution the embryos remain active for from one to three weeks.   On damp earth and in water which contains a large number of algæ a great many of the eggs hatch, but the young worms die very soon.

In a recent paper A. Martin [3] presents some very interesting results of work on the eggs of *Ascaris* from the calf, pig, horse, and dog.   He conclusively proves that the embryos of these ascarids hatch best in alkaline solutions, and that when developed eggs are introduced into the alimentary canal of an animal they pass through the stomach unaffected and only hatch after they have been subjected to the action of the alkaline juices in the intestine.   He finds also that none of the juices of the alimentary canal are able to digest the chitinous layers of the shell, that the embryos always emerge through a V-shaped opening which appears in the end of the shell, and that the shell passes out, undigested, with the fæces.   He is of the opinion that the hatching is due to stimulation of the embryos by the alkaline substances in the intestine and by the increase in temperature, and not to any action of the juices on the structure of the shell.

He found also, in the cases of the embryos of the calf and of the pig ascaris, that it was necessary for the embryos to be completely developed before being fed to an animal, or placed in

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[3] *Ann. d. Sci. natur.* (1913), Nos. 1 and 2.

artificial juices, at 37°, as any embryos which were not completely developed were always killed by the rise in temperature. The ascarids of the horse and dog were able to undergo their complete development and hatch in artificial pancreatic juice at a temperature of 37°.

My experiments in hatching the eggs of *Ascaris lumbricoides* have not been conclusive except on one point, and that is that the embryos must be completely developed before they are introduced into the alimentary canal. Artificial gastric and pancreatic juices have no apparent effect on the structure of the shell, but I have not been able to hatch the embryos with any degree of regularity. This is probably due to faulty technique, as in other respects the action of these eggs closely parallels Martin's results on the pig ascaris.

THE OCCURRENCE OF BACILLUS COLI COMMUNIS IN THE PERIPHERAL BLOOD OF MAN DURING LIFE [1]

By E. H. Ruediger

(From the Section of Sera and Prophylactics, Biological Laboratory, Bureau of Science, Manila, P. I.)

THREE TEXT FIGURES

While infections of the body tissues by *Bacillus coli communis* are extremely common, and while there is every reason to believe that the spread of the infection in a large number of the cases takes place by way of the blood stream, it is remarkable how few cases of blood infection with *Bacillus coli communis* during life have been reported. Brian [2] reported 6 cases and Tidy [3] reported 3 cases. All of the cases reported by Brian recovered, while of those reported by Tidy 2 had died and the third was still in the hospital practically unimproved when the report was written.

During routine bacteriologic examinations of blood at the Philippine General Hospital, 4 cases of blood infection by *Bacillus coli communis* came under my observation. Two of the cases proved fatal and 2 ended in recovery.

Case 1.—The patient, an adult Filipino, was moribund when the bacteriologic examination of the blood was made on July 10, 1912, and died within twenty-four hours. Ten cubic centimeters of blood were obtained and put into 200 cubic centimeters of citrated glucose bouillon. A profuse growth appeared, and there was gas production in the broth. On studying the organism further, the following biological and cultural characteristics were brought out: a short motile bacillus; in nutrient broth it produces uniform clouding, and a pellicle forms within a week. It grows readily on nutrient agar and produces gas in nutrient agar that contains glucose. Litmus milk is turned acid and is coagulated, and on potato it forms a large brownish growth. Diagnosis, *Bacillus coli communis*.

[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

[2] *Deutsch. Arch. f. klin. Med.* (1912), 106, 379.

[3] *Lancet*, London (1912), 2, 1500.

Case 2.—An adult Filipino was admitted to the Philippine General Hospital under the care of Dr. P. K. Gilman on August 27, 1914. The following diagnosis was made: Vesical calculus, chronic cystitis, chronic nephritis, amœbiasis, bronchopneumonia, ulcerative gastroenteritis, trichuriasis. A bacterio-
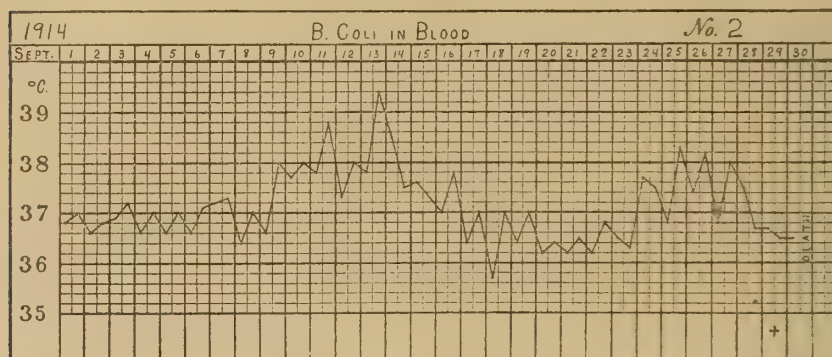

Fig. 1. Showing temperature of case 2.

logic examination of the blood was made on September 29, 1914, and a pure culture of *Bacillus coli communis* was obtained. The patient died within twenty-four hours after the blood was taken (see fig. 1, chart for case 2).

Case 3.—An adult Filipino was admitted to the Philippine General Hospital on September 7, 1914, complaining of orchitis. On September 11, 1914, he was operated upon for tuberculous
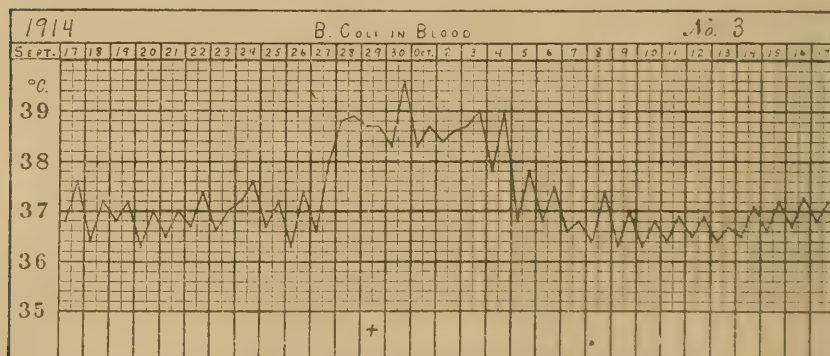

Fig. 2. Showing temperature of case 3.

epididymitis. On September 27, 1914, the temperature rose to 38° C., and on the following morning the thermometer registered 38°.8 C. A blood culture made on September 29, 1914, yielded a profuse growth of *Bacillus coli communis*. The temperature remained high and irregular and dropped sud-

denly from 39° C. to 36°.8 C. between the evening of October 4, 1914, and the morning of October 5, 1914.   After that the temperature remained low and recovery was rapid.

The serum obtained from patient 3 on October 5, 1914, agglutinated the organism obtained from his blood at a dilution of 1 : 200; the organisms from cases 1 and 2 and a stock strain of *Bacillus coli communis* and *Bacillus typhosus* were not agglutinated at a dilution of 1 : 50 (see fig. 2, chart for case 3).

Case 4.—An adult Filipina was admitted to the Philippine General Hospital on September 25, 1914, complaining of fever and general sick feeling, which began three days after she had been confined two weeks previous.   Examination showed a bloody discharge from the uterus.   On October 2, 1914, 10 cubic centimeters of blood obtained from a superficial vein in the arm were put into 200 cubic centimeters of citrate-glucose bouillon,
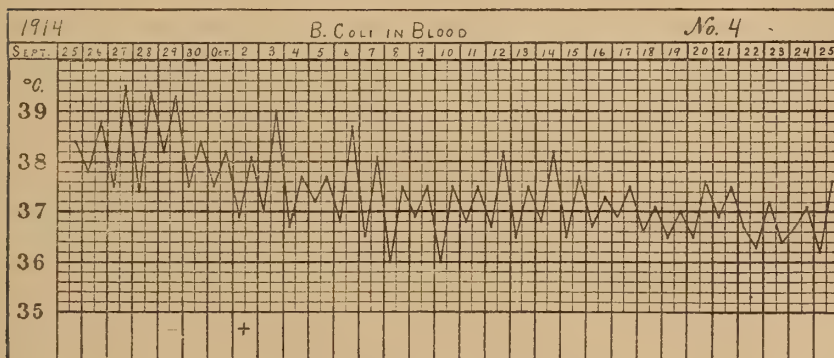

Fig. 3.   Showing temperature of case 4.

and a pure culture of *Bacillus coli communis* was obtained.   The temperature of the patient was very irregular and recovery was slow.

Blood serum obtained from patient 4 on November 5, 1914, agglutinated the organism obtained from her blood in a dilution of 1 : 400 in six hours.   The organisms from case 2 also was agglutinated in a dilution of 1 : 400 in six hours, while the organisms from cases 1 and 3 and a stock strain of *Bacillus coli communis* and of *Bacillus typhosus* were not agglutinated in a dilution of 1 : 25 (see fig. 3, chart for case 4).

CONCLUSIONS

1. Microörganisms corresponding in morphological and cultural characteristics to *Bacillus coli communis* may in certain cases be obtained from the peripheral blood of patients during life.

2. Invasion of the blood stream by such organisms is not necessarily terminal infection, as is shown by the large percentage of recoveries therefrom.

3. Such infection may be considered primary as is shown in case 3 here reported.

4. As a peripheral infection like that of case 4 the bacillus may enter the blood stream through the infected uterus.

5. The agglutination test shows that these organisms differ from one another.

ILLUSTRATIONS

THE PREPARATION OF TETANUS ANTITOXIN [1]

By E. H. RUEDIGER

*(From the Section of Sera and Prophylactics, Biological Laboratory,
Bureau of Science, Manila, P. I.)*

EIGHTY-FIVE TEXT FIGURES

Notwithstanding the fact that tetanus antitoxin is extensively used in practically all countries of the world, very little has been written about its preparation in recent years.

Eisler and Pribram [2] advise the injection of tetanus toxin and iodine trichloride for the first three months of the time that a horse is being immunized against tetanus toxin. This is followed by another three months' treatment with tetanus toxin alone. Although weakening the tetanus toxin by mixing it with iodine trichloride saves the lives of many serum horses and is almost indispensable when tetanus antitoxin is not available, it is now rarely used; the horses can be, and now usually are, fortified with the antitoxin.

In order to obtain good antitoxin, good toxin is indispensable. Good toxin usually can be obtained by growing suitable tetanus bacilli in glucose broth under anaërobic conditions. In my experience the following procedure has given fairly good results:

To 500 grams of chopped lean beef add 1,000 cubic centimeters of distilled water and boil for one hour. Enough water should be added to allow for evaporation. Allow the infusion to cool; strain and add the following: Witte's pepton, 20 grams; sodium chloride, 5 grams; glucose, 10 grams.

Prepare the broth in the usual way and with sodium hydrate solution reduce the acidity to about 0.5 per cent normal acid. Pass the broth through a paper filter, sterilize it in the autoclave, cool it rapidly in running water, inoculate with tetanus bacilli, and incubate it under hydrogen at a temperature of from 36° C. to 37° C. for from seven to ten days (rarely fourteen days). After having been incubated sufficiently long, the broth is rendered germ-free by passing it through a sterile germ-proof filter. Phenol, in the proportion of 0.5 cubic centimeter per 100 cubic centimeters of filtrate, may be added. The reaction of the filtrate will be about 2 per cent normal acid. This should be reduced to practically neutral by adding sodium hydrate solution. An acid filtrate produces severe local reaction on subcutaneous injection, while after the injection

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of neutral or nearly neutral filtrate (not more than 0.5 per cent normal acid) the local reaction is comparatively mild.

The toxin obtained by this method will usually be such that 0.0001 (1/10,000) cubic centimeter when injected under the skin of a 300-gram guinea pig will prove fatal within five days. Toxin of which 0.00005 (1/20,000) cubic centimeter killed the guinea pig within five days has frequently been obtained, and in one instance the filtrate was such that 0.00002 (1/50,000) cubic centimeter killed a 300-gram guinea pig within five days.

Recently good toxin has been obtained by the method described by Ivan Hall.[3]   His method is as follows:

1. Mix distilled water .... 1,000 c.c.
    NaCl (C. P.) ....   5 gr.
    Pepton (Witte) ....   10 gr.
    Beef Extract (Armour's soluble beef)...................................   5 gr.
    Dextrose (Commercial) ....   10 gr.
    MgCO₃ (finely powdered commercial)...................................   5 gr.
2. Determine gross weight.
3. Dissolve by boiling and stirring.
4. Restore the original weight with distilled water.
5. Cool overnight to precipitate the phosphates.
6. Filter through coarse paper. Reaction should now be found faintly alkaline to phenolphthalein.
7. Add 2 gm. $MgCO_3$ and mix thoroughly.
8. Pour into Florence flasks to shoulder.
9. Cover to a depth of 2 c.m. with hydrocarbon oil (Langley & Michaels, San Francisco).
10. Sterilize in the Arnold 3 successive days, 40 minutes each time.

On the following pages are reported the methods of immunization and the results obtained from 8 horses at the Bureau of Science, Manila, P. I.

HORSE 1

Horse 1 in this series was given about 1,000 units of tetanus antitoxin [4] on June 4, 1911, and another dose of about 1,000 units on June 23, 1911. Tetanus toxin was given at short intervals, and the quantity was rapidly increased.

During the first three months 26 injections were given, and at the last injection the horse received 600 cubic centimeters of toxic filtrate. It became evident that the injections were being pushed too rapidly, so the horse was allowed to recuperate. On

[3] *Univ. Calif. Publ. Path.* (1913), 2, 98.

[4] Unit of tetanus antitoxin refers to the standard unit of the United States of America. The quantity of antitetanic serum which neutralizes the test dose (approximately 100 minimal lethal doses) contains 0.01 of a unit of antitoxin, hence a unit may be said to neutralize 1,000 minimal lethal doses of toxin tested on guinea pigs weighing 350 grams each.

September 11, 1911, another injection of 750 cubic centimeters was given. The serum was tested for antitoxin and was found to contain 150 units per cubic centimeter. On September 20, 1911, the horse was bled 3 liters and three days later was bled 2 liters.

Beginning with October 2, 1911, the injections were given at intervals of a week and the doses were increased more gradually. A test sample of blood taken in the latter part of October contained 350 units of antitoxin per cubic centimeter of serum. On November 1, 1911, the horse was bled 3 liters. The injections were continued at intervals of a week and on November 30, 1911, 3 liters of blood were withdrawn. The serum contained 500 units of antitoxin per cubic centimeter. After this, the antitoxin content in the serum rose more slowly, testing 600 units per cubic centimeter on December 31, 1911, and the horse had lost much flesh. Still expecting to drive the antitoxin content higher, the injections were continued; but instead of rising, the antitoxin content began to fall. On January 25, 1912, the horse was bled 3 liters and the serum tested 500 units per cubic centimeter.

This horse received a total of 8,856 cubic centimeters of toxin, was bled 14 liters, and furnished about 5,600 cubic centimeters of serum, or 1,920,000 units of antitoxin. Deducting 50 per cent as allowance made for deterioration and accidental losses, we can count on marketing 960,000 units (see figs. 1 to 9, charts 1–A to 1–I, for horse 1).

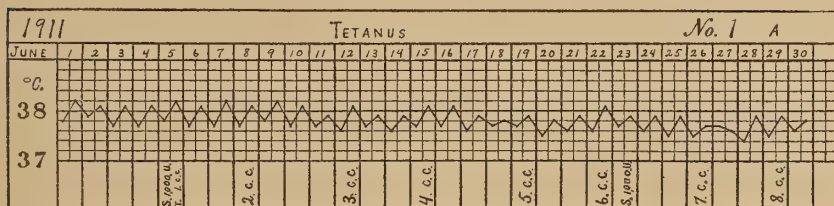FIG. 1. Temperature chart of horse 1 for June, 1911.

FIG. 2. Temperature chart of horse 1 for July, 1911.

FIG. 3.   Temperature chart of horse 1 for August, 1911.

FIG. 4.   Temperature chart of horse 1 for September, 1911.

FIG. 5.   Temperature chart of horse 1 for October, 1911.

FIG. 6.   Temperature chart of horse 1 for November, 1911.
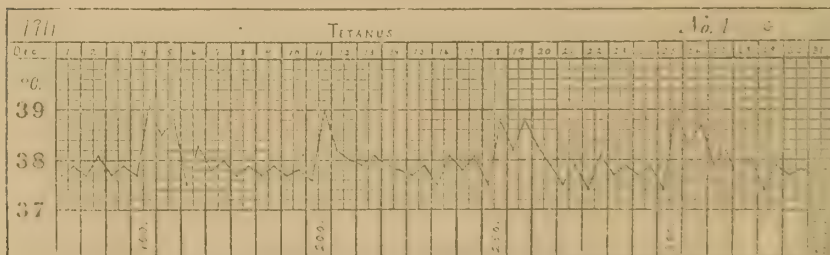
FIG. 7.   Temperature chart of horse 1 for December, 1911.

FIG. 8.   Temperature chart of horse 1 for January, 1912.

FIG. 9. Chart showing the antitoxin curve for horse 1. Units per cubic centimeter by months.

HORSE 2

The immunization of horse 2 was begun on July 2, 1911.   It
was fortified with 2,500 units of tetanus antitoxin, 750 units
of which were given on July 2, 1911, 750 units on July 15, 1911,

and 1,000 units on August 1, 1911. The injection of toxin was begun with 0.01 cubic centimeter filtrate given at intervals of three or four days, and the doses were rapidly increased. During the first three months 21 injections were given, and at the last injection 1,000 cubic centimeters of toxic filtrate were given. On September 27, 1911, the serum was tested; it contained less than 50 units of antitoxin per cubic centimeter.

After September the doses were reduced and the injections were given at intervals of a week. On November 10, 1911, the horse was bled 3 liters, and the serum contained 75 units per cubic centimeter. Three liters of blood were withdrawn on December 21, 1911; the serum obtained tested 100 units per cubic centimeter. The injections were continued at intervals of a week; on February 23, 1912, the horse was bled 4 liters, and on February 29, 1912, it was bled 5 liters. The serum contained 150 units per cubic centimeter.

Horse 2 received a total of 8,806 cubic centimeters of tetanus toxin; it was bled 15 liters and produced about 6 liters of antitoxic serum or 750,000 units of antitoxin. Deducting 50 per cent of antitoxin as allowance made for deterioration and other losses, we have left 375,000 units for marketing.

At this time there was very little demand for antitetanic serum or the horses would have been bled more, as will be seen later (see figs. 10 to 18, charts 2–A to 2–I, for horse 2).
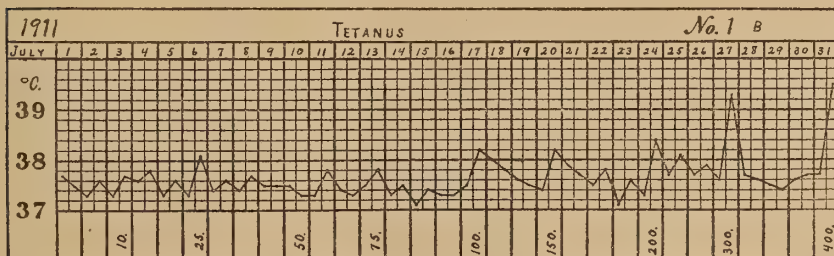

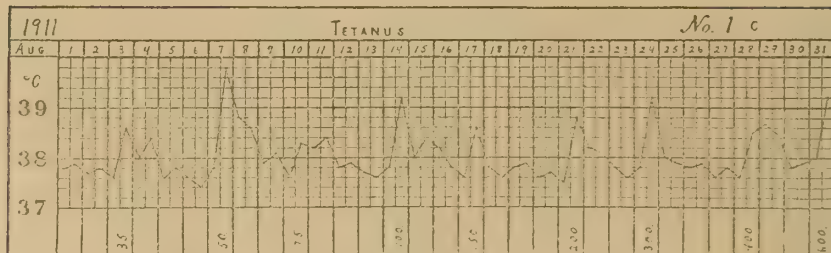Fig. 10. Temperature chart of horse 2 for July, 1911.

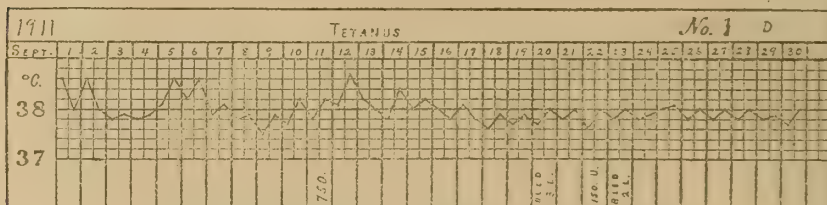Fig. 11. Temperature chart of horse 2 for August, 1911.

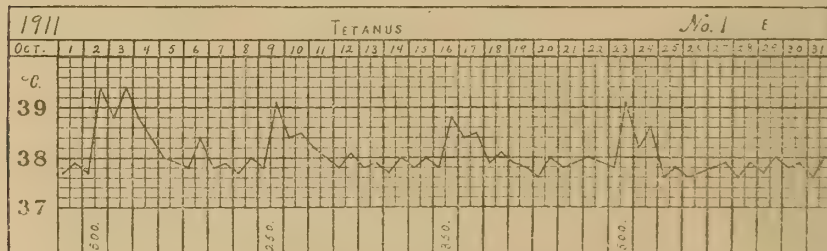FIG. 12.   Temperature chart of horse 2 for September, 1911.

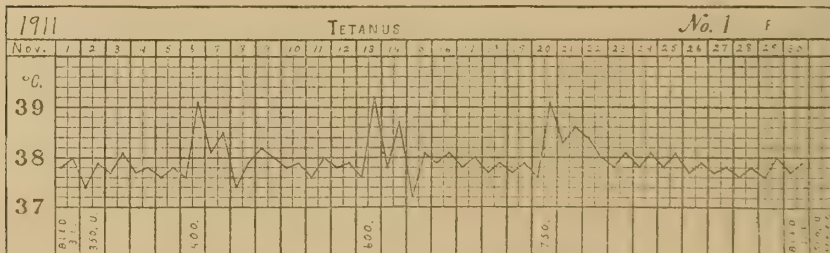FIG. 13.   Temperature chart of horse 2 for October, 1911.

FIG. 14.   Temperature chart of horse 2 for November, 1911.

FIG. 15.   Temperature chart of horse 2 for December, 1911.

FIG. 16. Temperature chart of horse 2 for January, 1912.
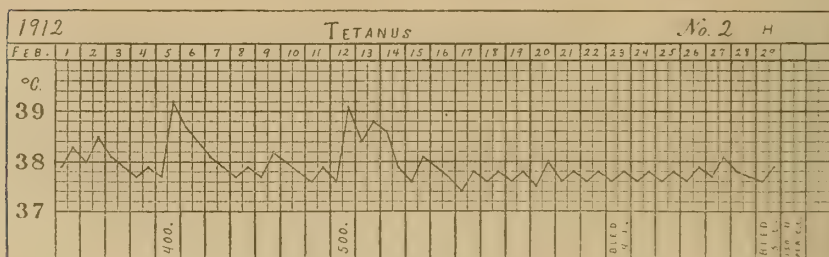
FIG. 17. Temperature chart of horse 2 for February, 1912.
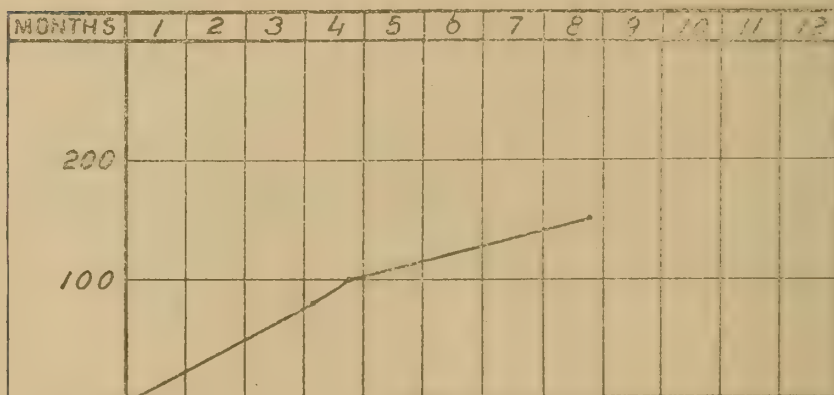
FIG. 18. Chart showing the antitoxin curve for horse 2. Units per cubic centimeter by months.

HORSE 3

Immunization of horse 3 was begun at the same time as that of horse 2, and the two received practically the same treatment. This horse produced much stronger antitoxin than did horse 2; in February, 1912, when the serum of horse 2 contained 150 units of antitoxin per cubic centimeter, the serum of horse 3

contained 400 units of antitoxin per cubic centimeter. The antitoxin content reached its highest mark at the end of the eighth month.

Horse 3 received a total of 8,806 cubic centimeters of tetanus toxin; it was bled 15 liters and produced about 6 liters of antitoxic serum or 1,400,000 units of antitoxin. Deducting from the antitoxin 50 per cent as allowance made for deterioration and accidental losses, we can count on marketing 720,000 units (see figs. 19 to 27, charts 3–A to 3–I, for horse 3).
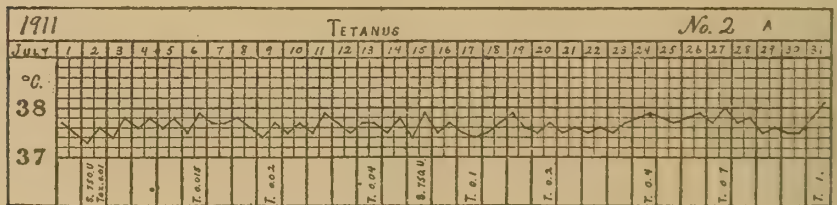

FIG. 19. Temperature chart of horse 3 for July, 1911.
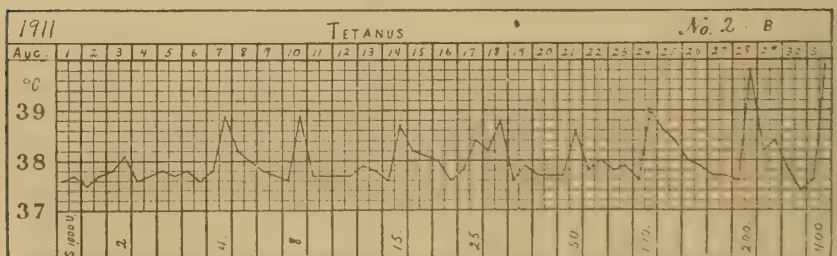

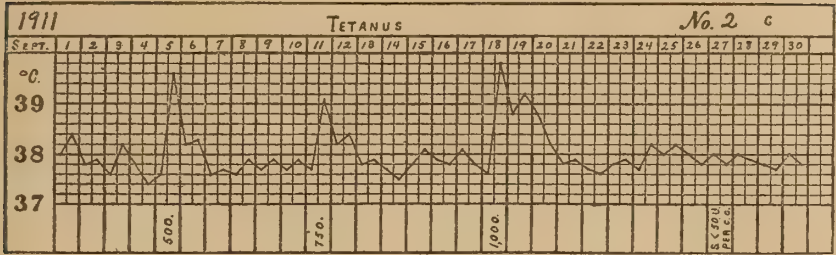FIG. 20. Temperature chart of horse 3 for August, 1911.

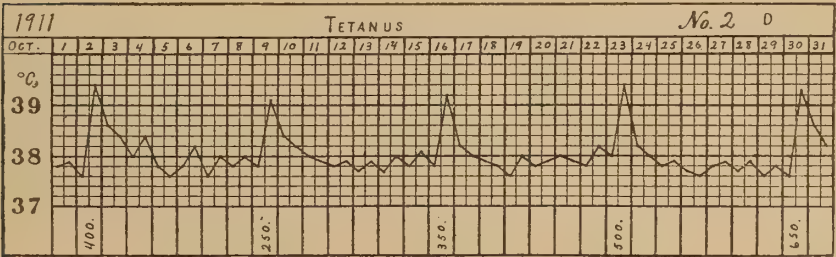FIG. 21. Temperature chart of horse 3 for September, 1911.
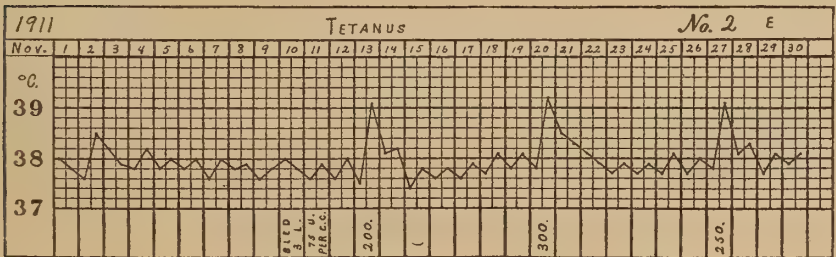
FIG. 22.   Temperature chart of horse 3 for October, 1911.

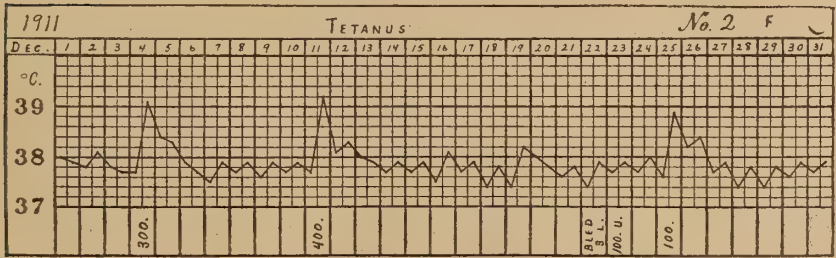FIG. 23.   Temperature chart of horse 3 for November, 1911.

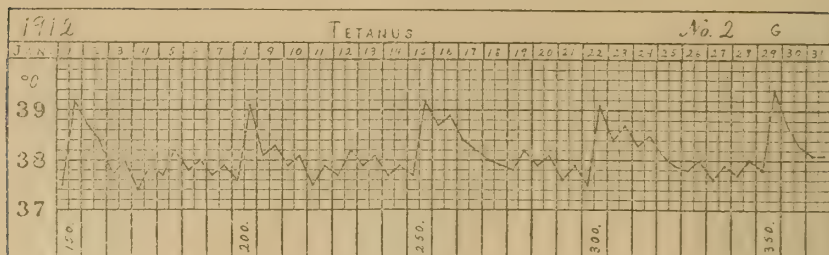FIG. 24.   Temperature chart of horse 3 for December, 1911.
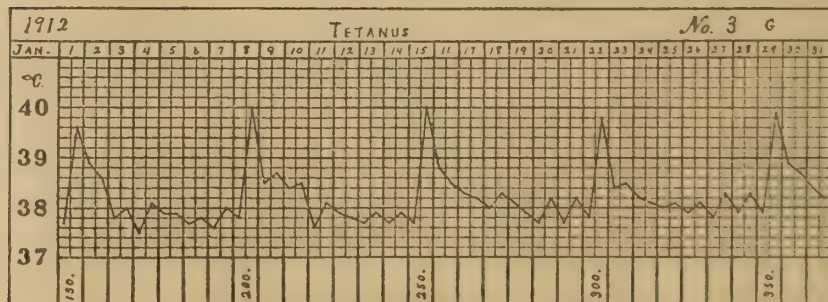
FIG. 25.   Temperature chart of horse 3 for January, 1912.

FIG. 26.   Temperature chart of horse 3 for February, 1912.
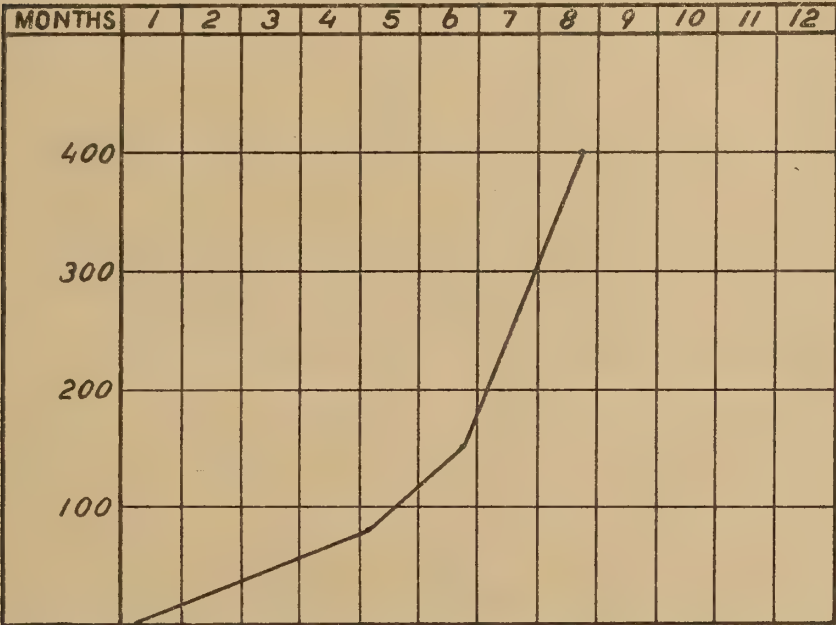
FIG. 27.   Chart showing the antitoxin curve for horse 3. Units per cubic centimeter by months.

HORSES 4 AND 5

On June 1, 1913, the immunization of horses 4 and 5 was begun.   Each horse was fortified with 2,500 units of tetanus antitoxin, 1,500 units being given on June 1, 1913, and 1,000 units on July 7, 1913.   Beginning with a dose of 2 cubic centimeters, injections of toxin were made at intervals of one week. The doses were increased to 1,000 cubic centimeters in four months and one week.   The serums contained 75 units of antitoxin per cubic centimeter, and each horse was bled; horse 4 was bled 8 liters, and horse 5, 10 liters.   After this bleeding smaller doses of toxin were given.   In the latter part of November, 1913, the serum of horse 4 contained 300 units per cubic

centimeters and 11 liters of blood were withdrawn, about 40 per cent of which was serum. Horse 5 was bled 12 liters in the first week of December, 1913, which yielded about 40 per cent of serum that contained 300 units of antitoxin per cubic centimeter. The injections of toxin were again continued. On January 21, 1914, horse 4 was bled 6 liters; the serum contained 300 units of antitoxin per cubic centimeter. Horse 5 was bled 10 liters—5 liters on January 23, 1914, and 5 liters on January 27, 1914. The serum obtained contained 250 units per cubic centimeter. In the middle of March, 1914, the serum of horse 4 contained 300 units of antitoxin per cubic centimeter and the horse was bled 8 liters. The serum of horse 5 contained 350 units of antitoxin per cubic centimeter, and 10 liters of blood were withdrawn on March 23. Horse 4 received one more injection of toxin and was bled to death on March 31, 1914. At the last bleeding 14 liters of blood were obtained which yielded about 40 per cent of serum, testing 225 units per cubic centimeter. The antitoxin content in the serum of horse 4 reached its highest recorded mark in the sixth month and was maintained until death, ten months after the beginning of immunization.

Horse 4 received in all 14,633.5 cubic centimeters of tetanus toxin and was bled 47 liters. It produced about 18.8 liters of antitetanic serum or 4,500,000 units of tetanus antitoxin. Deducting from the antitoxin 50 per cent as allowance made for deterioration and for accidental losses, we have left 2,250,000 units for marketing (see figs. 28 to 38, charts 4–A to 4–K, for horse 4).

Fig. 28. Temperature chart of horse 4 for June, 1913.

Fig. 29. Temperature chart of horse 4 for July, 1913.
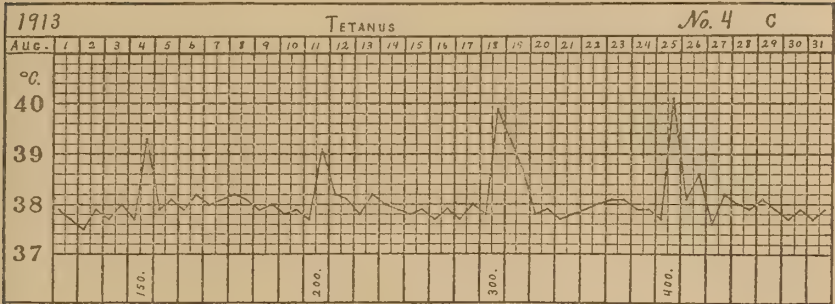
FIG. 30.   Temperature chart of horse 4 for August, 1913.
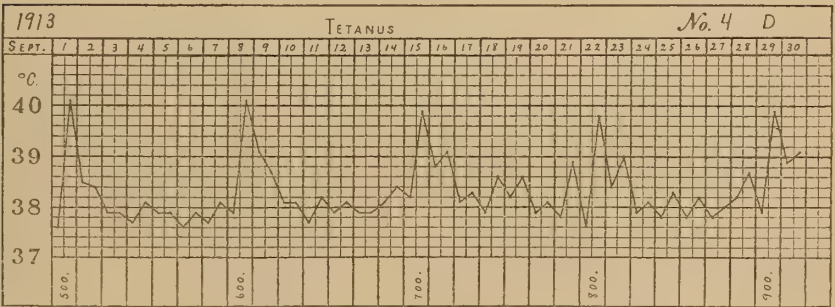
FIG. 31.   Temperature chart of horse 4 for September, 1913.

FIG. 32.   Temperature chart of horse 4 for October, 1913.

FIG. 33.   Temperature chart of horse 4 for November, 1913.

Fig. 34. Temperature chart of horse 4 for December, 1913.

Fig. 35. Temperature chart of horse 4 for January, 1914.

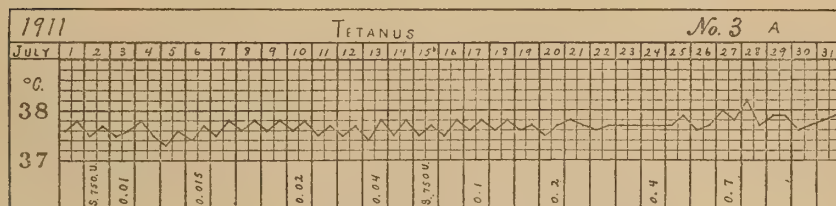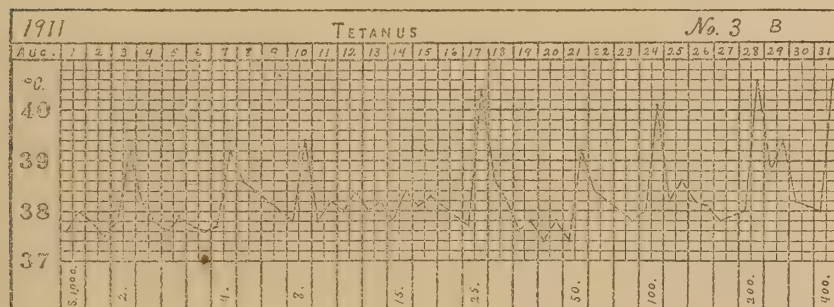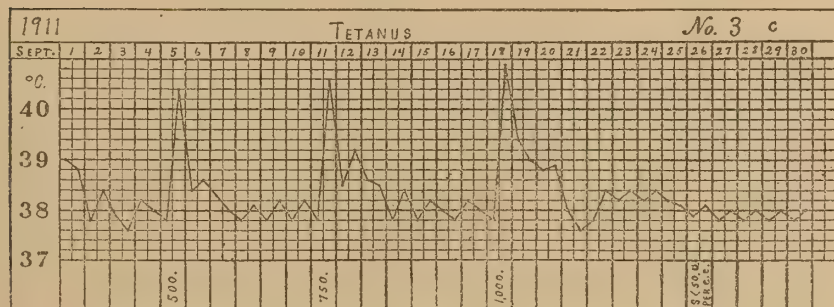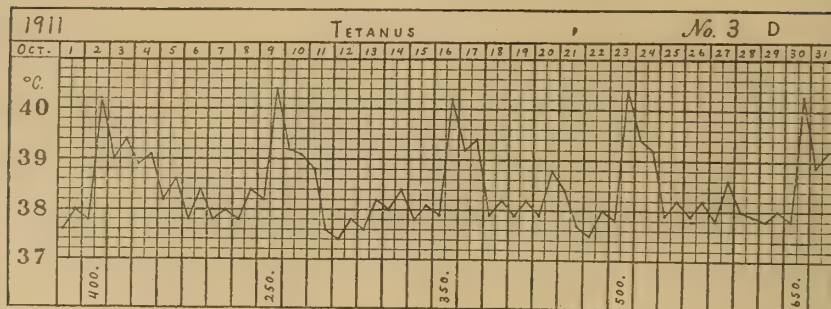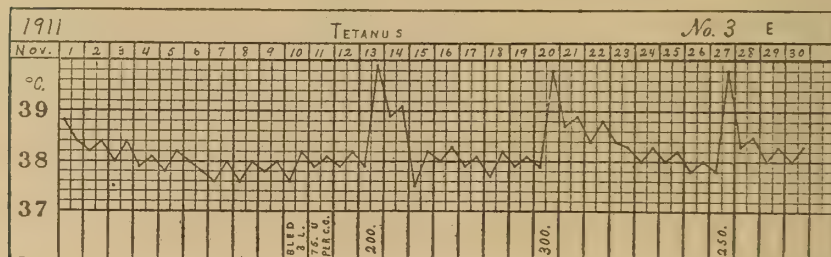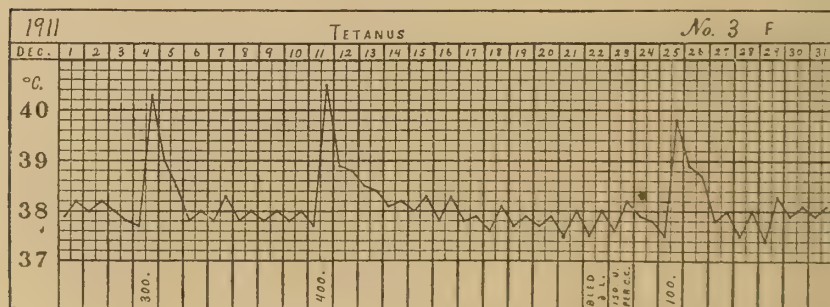Fig. 36. Temperature chart of horse 4 for February, 1914.
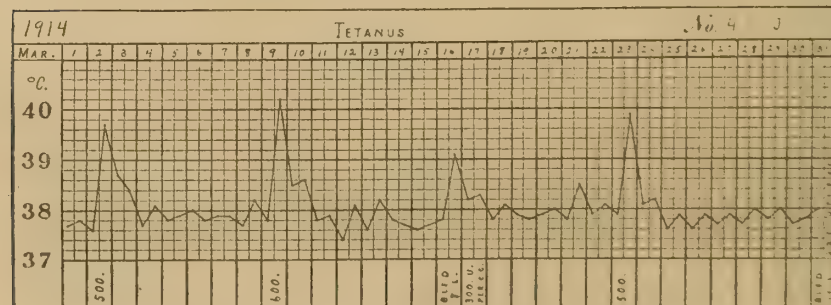
Fig. 37. Temperature chart of horse 4 for March, 1914.
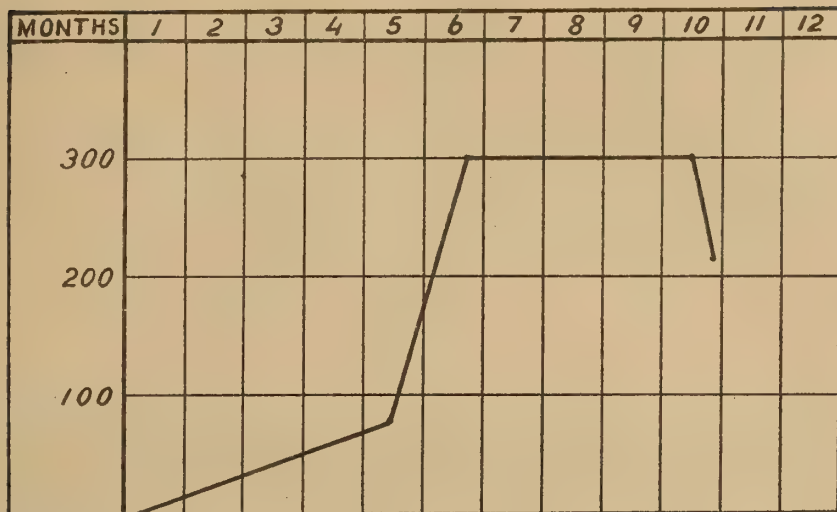
Fig. 38. Chart showing the antitoxin curve for horse 4. Units per cubic centimeter by months.

The injections of toxin were continued on horse 5. In the middle of May, 1914, the serum was tested and was found to contain only about 125 units of antitoxin per cubic centimeter. On May 19, 1914, the horse was bled 6 liters and after that was used for other purposes.

The antitoxin content of the serum of horse 5 reached the highest recorded mark in the seventh month, fell about 50 units per cubic centimeter in the eighth month, and rose again to, or a little over, the previous height in the tenth month, and after that it began to fall.

Horse 5 received a total of 18,433.5 cubic centimeters of tetanus toxin and was bled 48 liters. During one year this horse produced about 19.2 liters of antitetanic serum or 4,430,000 units of tetanus antitoxin. After deducting 50 per cent for loss, we have left 2,215,000 units of antitoxin (see figs. 39 to 51, charts 5–A to 5–M, for horse 5).

Fig. 39. Temperature chart of horse 5 for June, 1913.

FIG. 40.   Temperature chart of horse 5 for July, 1913.

FIG. 41.   Temperature chart of horse 5 for August, 1913.

FIG. 42.   Temperature chart of horse 5 for September, 1913.

FIG. 43.   Temperature chart of horse 5 for October, 1913.

Fig. 44.   Temperature chart of horse 5 for November, 1913.

Fig. 45.   Temperature chart of horse 5 for December, 1913.

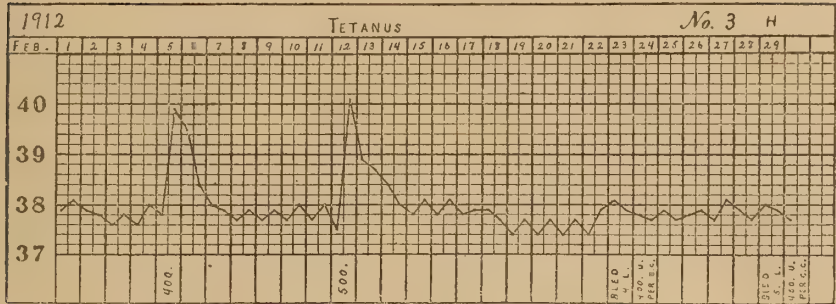Fig. 46.   Temperature chart of horse 5 for January, 1914.

Fig. 47.   Temperature chart of horse 5 for February, 1914.

FIG. 48. Temperature chart of horse 5 for March, 1914.

FIG. 49. Temperature chart of horse 5 for April, 1914.
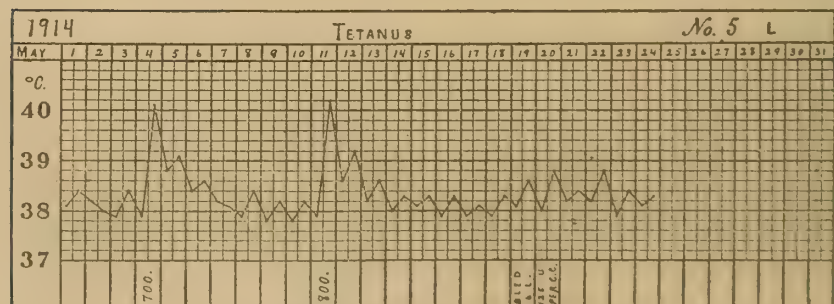
FIG. 50. Temperature chart of horse 5 for May, 1914.
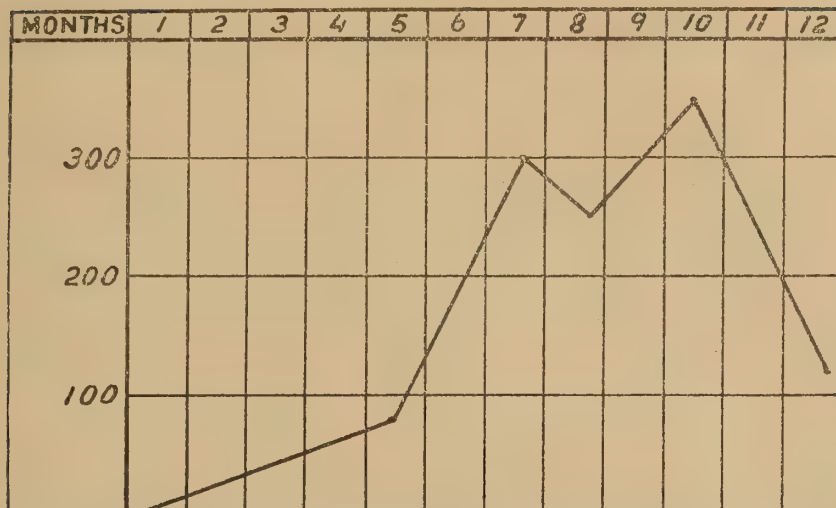
Fig. 51. Chart showing the antitoxin curve for horse 5. Units per cubic centimeter by months.

HORSE 6

Immunization of horse 6 was begun on November 10, 1913. It was fortified with 2,000 units of tetanus antitoxin, 1,000 units of which were injected on November 10, 1913, and 1,000 units, on November 24, 1913. Beginning with a dose of 1 cubic centimeter, tetanus toxin was injected at intervals of a week and the doses were increased more gradually than had been the practice previously.   A test bleeding was made on April 6, 1914, and the serum was found to contain 250 units of antitoxin per cubic centimeter.   The horse was bled 6 liters on April 14, 1914, and again 6 liters on April 18, 1914, the serum testing a little more than 250 units of antitoxin per cubic centimeter each time. The injections of toxin were continued.   Eight liters of blood were withdrawn on May 26, 1914, from which about 3.2 liters of serum were obtained, which tested about 300 units per cubic centimeter.   Four injections of toxin were given during June, 1914.   On June 29, 1914, the horse was bled 5 liters; the serum contained 500 units of antitoxin per cubic centimeter. On July 3, 1914, the horse was bled to death; 12 liters of blood were obtained this time.   The serum contained more than 450, but less than 500, units of antitoxin per cubic centimeter.

Horse 6 received in all 9,578.5 cubic centimeters of tetanus toxin and furnished 37 liters of blood.   It produced about 14.8 liters of antitetanic serum or 5,320,000 units of tetanus anti-

toxin. Deducting 50 per cent from the antitoxin as loss, we have left 2,660,000 units of antitoxin for the market (see figs. 52 to 61, charts 6–A to 6–J, for horse 6).

FIG. 52. Temperature chart of horse 6 for November, 1913.
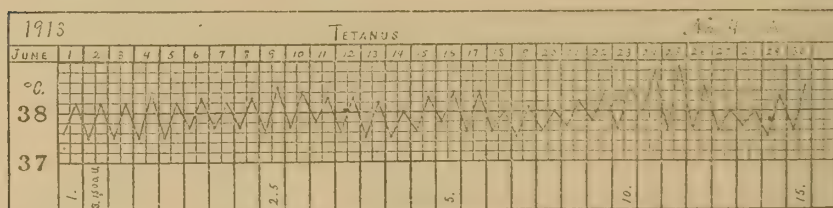

FIG. 53. Temperature chart of horse 6 for December, 1913.

FIG. 54. Temperature chart of horse 6 for January, 1914.

FIG. 55. Temperature chart of horse 6 for February, 1914.
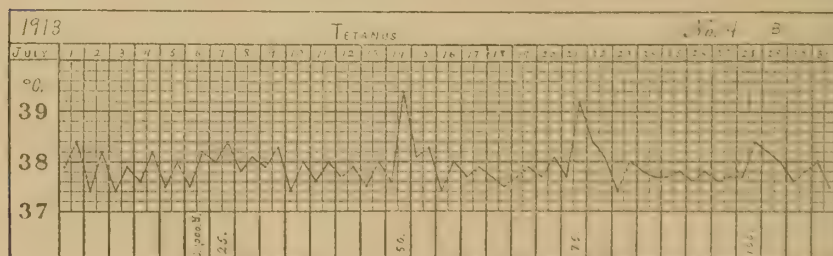
Fig. 56. Temperature chart of horse 6 for March, 1914.

Fig. 57. Temperature chart of horse 6 for April, 1914.
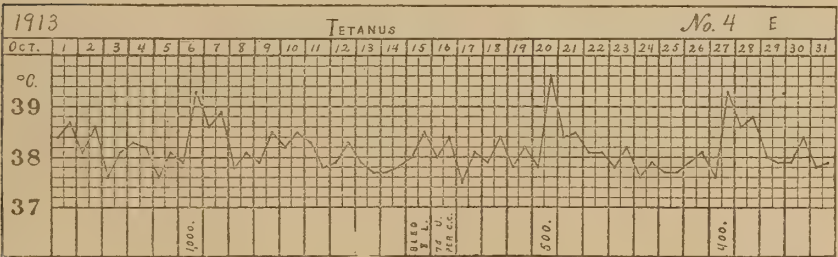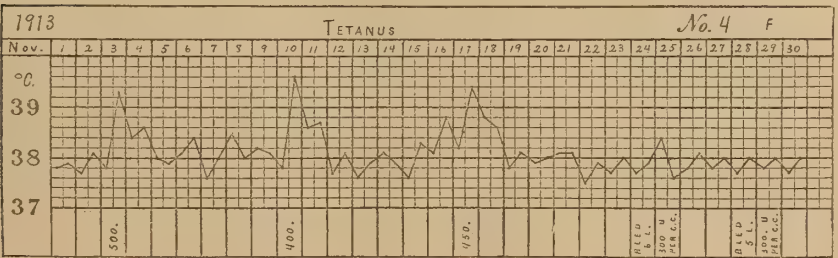
Fig. 58. Temperature chart of horse 6 for May, 1914.

Fig. 59. Temperature chart of horse 6 for June, 1914.

FIG. 60. Temperature chart of horse 6 for July, 1914.
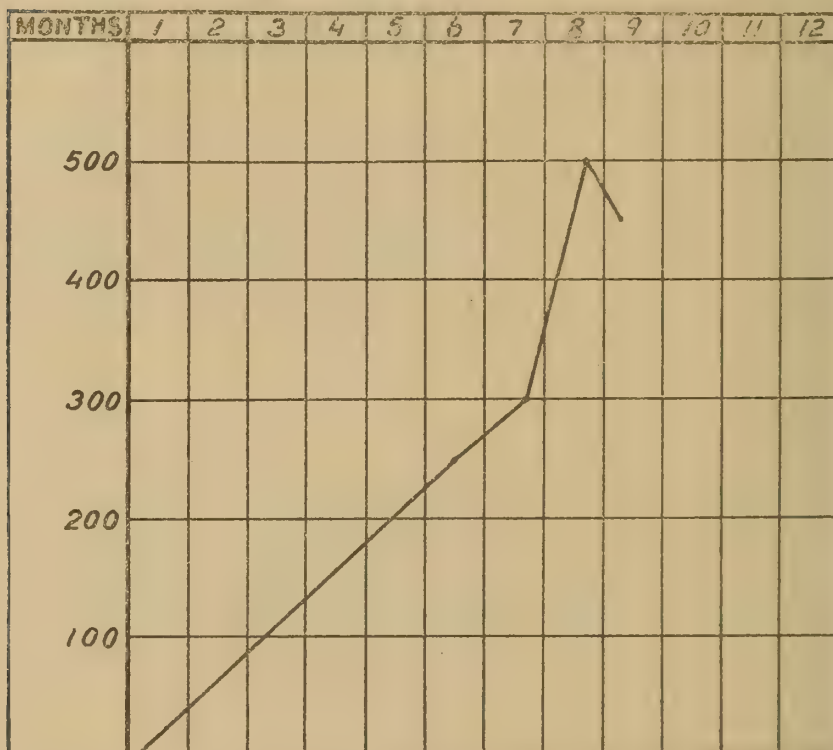
FIG. 61. Chart showing the antitoxin curve for horse 6. Units per cubic centimeter by months.

HORSE 7

The treatment of horse 7 was begun on November 10, 1913. As a prophylactic 2,000 units of tetanus antitoxin were given, 1,000 units on November 10, 1913, and 1,000 units on November 24, 1913.[5]

The injection of tetanus toxin was begun with a dose of 1

[5] Although for the want of space antitoxin and toxin are recorded under different dates on the charts, these were given simultaneously. 1,000 units of antitoxin and 1 cubic centimeter of toxin on November 10, 1913, and 1,000 units of antitoxin and 3 cubic centimeters of toxin on November 24, 1913.

cubic centimeter; the doses were gradually increased and given at intervals of a week. In three months the dose was increased to 100 cubic centimeters. On March 9, 1914, the serum contained 250 units of tetanus antitoxin per cubic centimeter. In April, 1914, the horse was bled 11 liters—6 liters on April 6 and 5 liters on April 10. The serum tested 500 units of antitoxin per cubic centimeter. The injections of toxin were continued. Five liters of blood were withdrawn on June 2, 1914, the serum of which contained 700 units of antitoxin per cubic centimeter. In July, 1914, the horse was bled four times, 5, 12, 10, and 10 liters being obtained; the serum obtained tested 700, 700, 600, and 500 units, respectively, per cubic centimeter. Three injections of tetanus toxin were given, then 19 liters of blood were withdrawn: 10 liters on August 11, 1914, and 9 liters on August 14, 1914. The serum contained 500 units and 400 units, respectively. Nine liters of blood withdrawn on September 1, 1914, yielded 3.5 liters of serum which contained 250 units of antitoxin per cubic centimeter. Nine liters of blood obtained on September 4, 1914, produced 4.4 liters of serum with 225 units of tetanus antitoxin per cubic centimeter. A third bleeding of 5 liters on September 7, 1914, yielded 2.5 liters of serum with about 200 units of antitoxin per cubic centimeter. On September 9, 1914, the horse was bled to death. Nineteen liters of blood were obtained, which yielded 10 liters of serum that contained 150 units of tetanus antitoxin per cubic centimeter.

The antitoxin content of the serum of horse 7 reached its highest recorded mark in seven months. This horse received a total of 14,619.5 cubic centimeters of tetanus toxin and furnished 114 liters of blood. It produced 51.7 liters of antitetanic serum, or 21,315,000 units of tetanus antitoxin. If we consider 50 per cent of the antitoxin as lost through deterioration and other accidental causes, we can still count on marketing 10,657,500 units of tetanus antitoxin produced by 1 horse in ten months (see figs. 62 to 73, charts 7–A to 7–L, for horse 7).
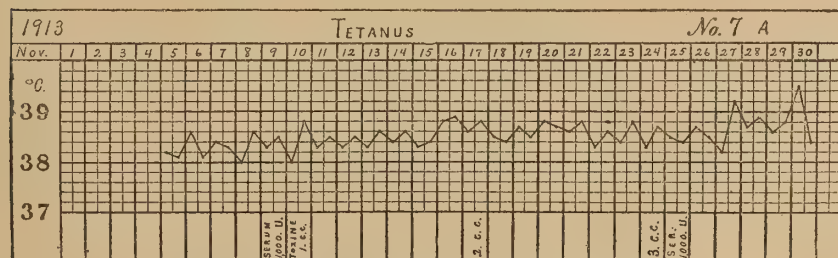
FIG. 62. Temperature chart of horse 7 for November, 1913.
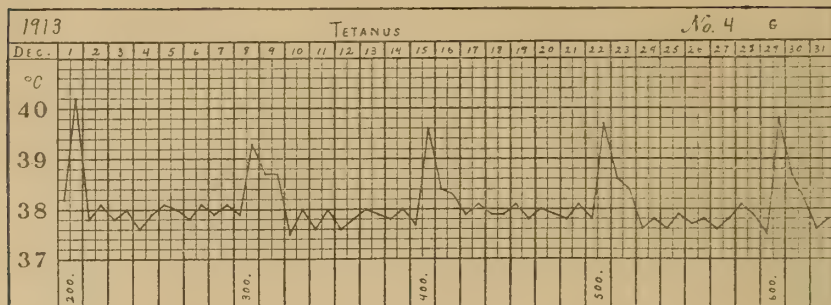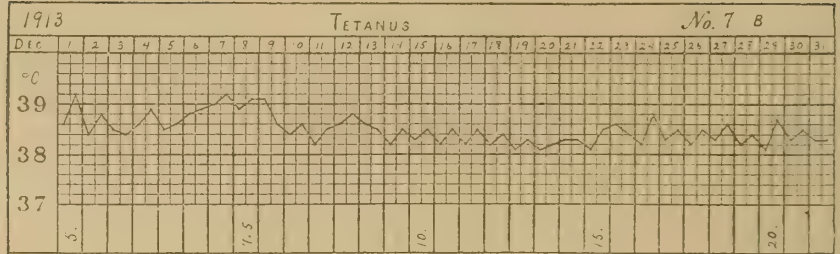
FIG. 63. Temperature chart of horse 7 for December, 1913.
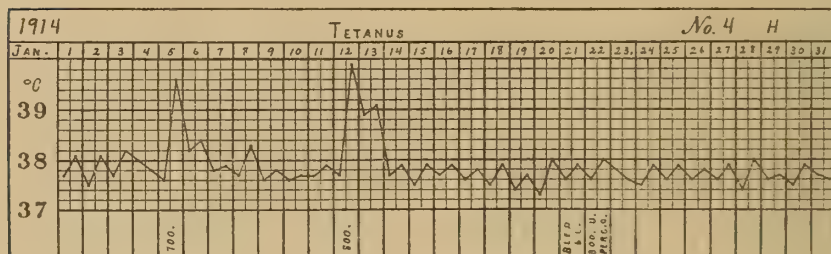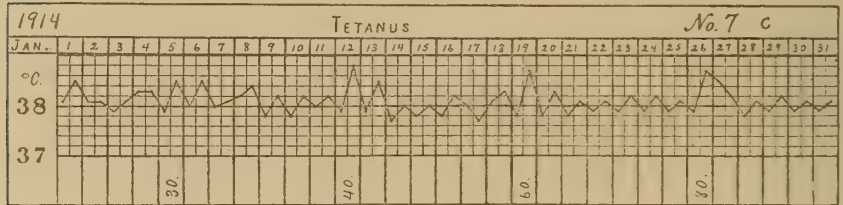

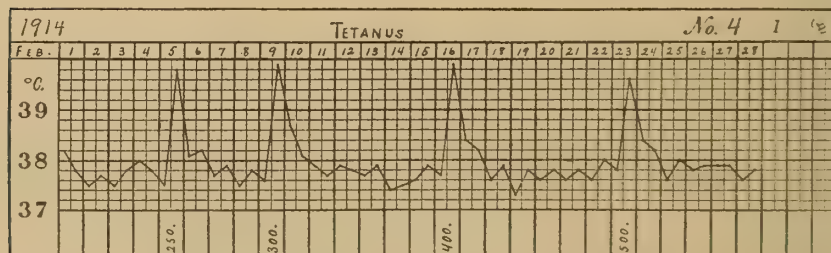FIG. 64. Temperature chart of horse 7 for January, 1914.
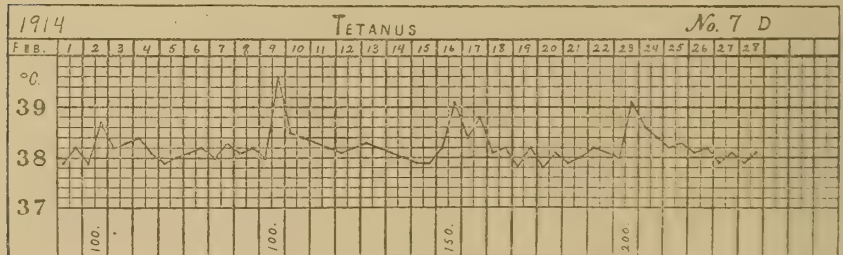
FIG. 65. Temperature chart of horse 7 for February, 1914.
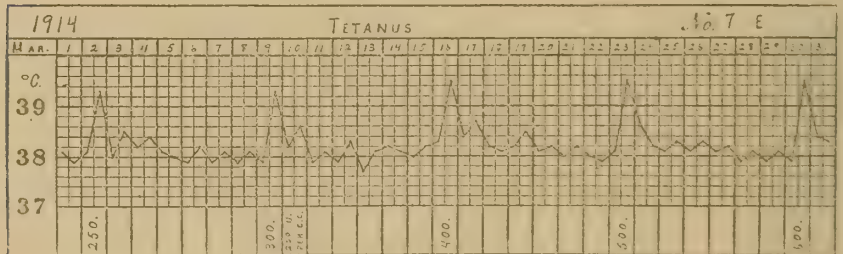
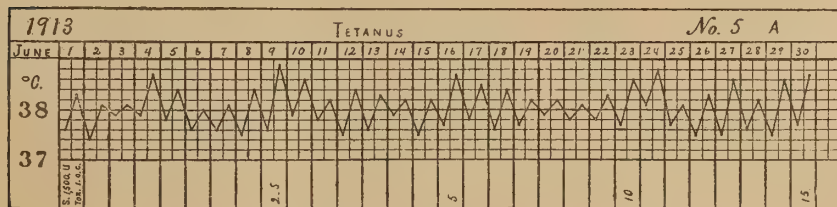FIG. 66. Temperature chart of horse 7 for March, 1914.

FIG. 67. Temperature chart of horse 7 for April, 1914.
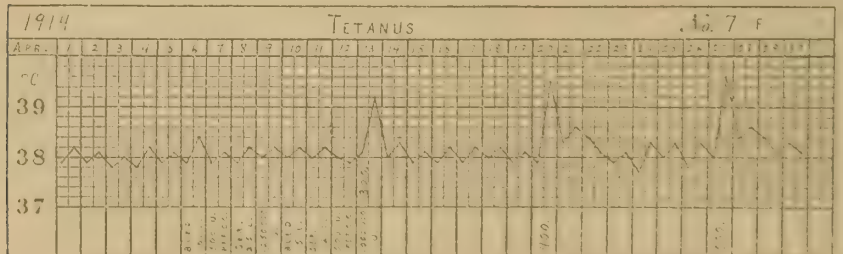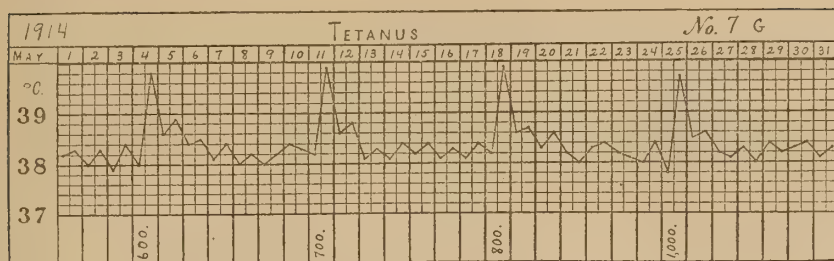
Fig. 68. Temperature chart of horse 7 for May, 1914.
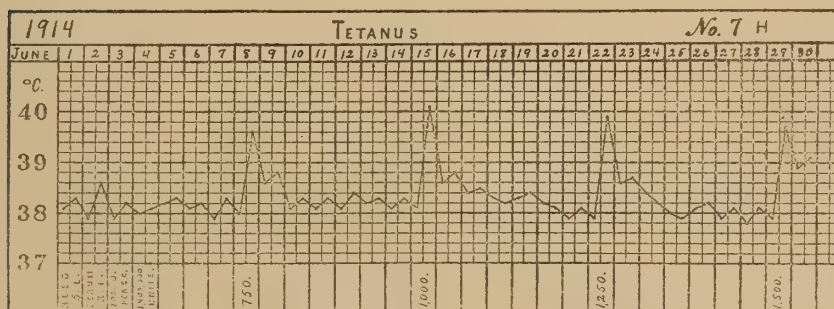
Fig. 69. Temperature chart of horse 7 for June, 1914.
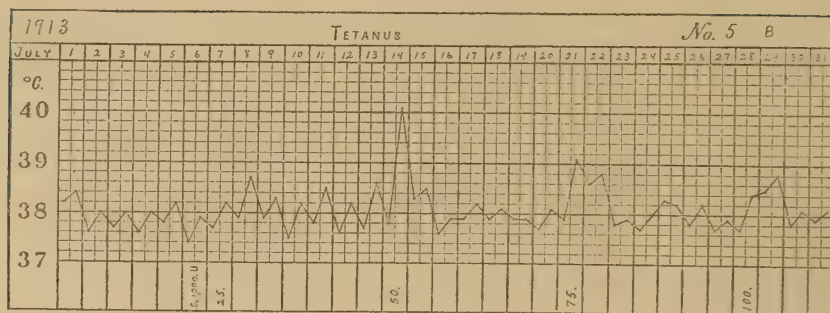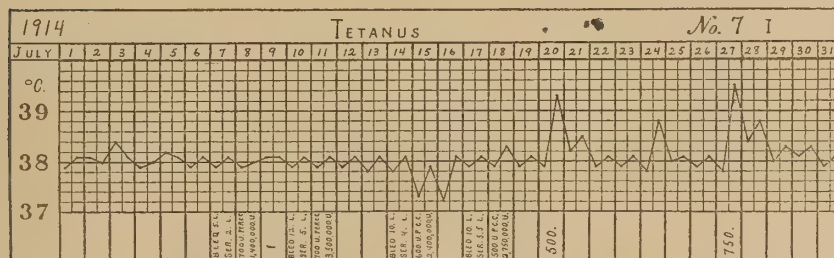
Fig. 70. Temperature chart of horse 7 for July, 1914.
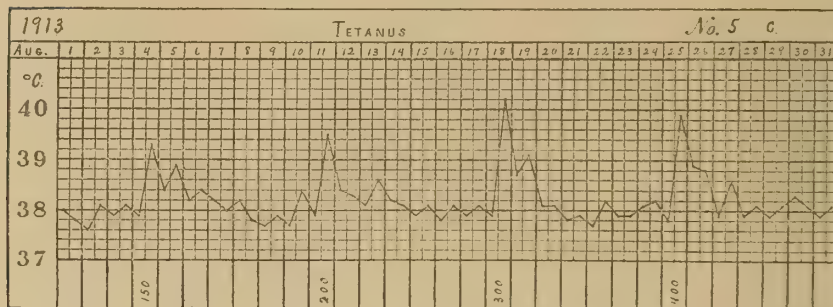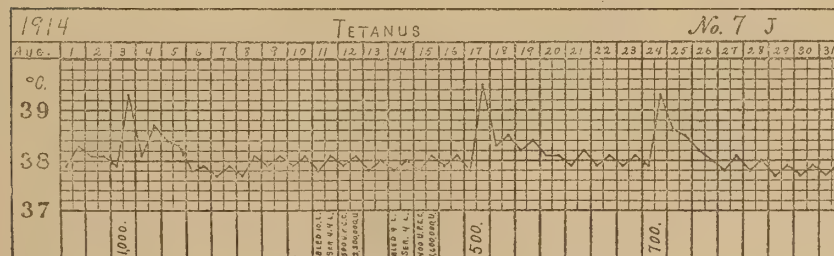
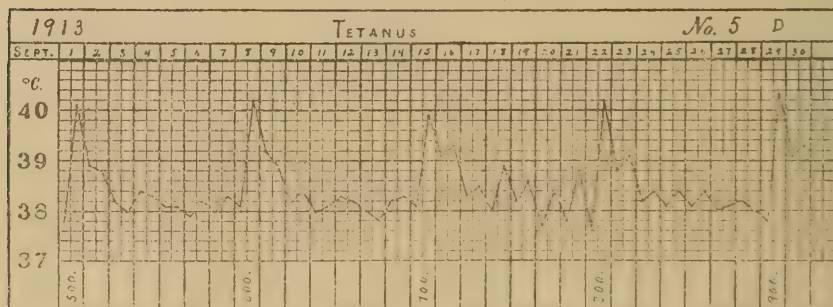Fig. 71. Temperature chart of horse 7 for August, 1914.
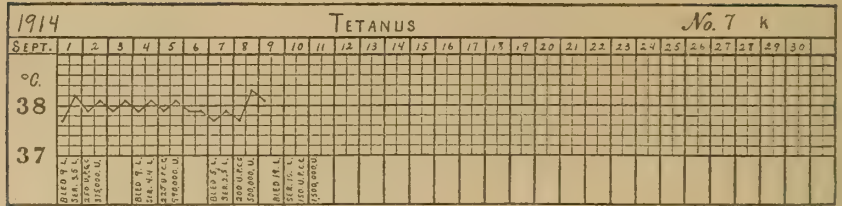
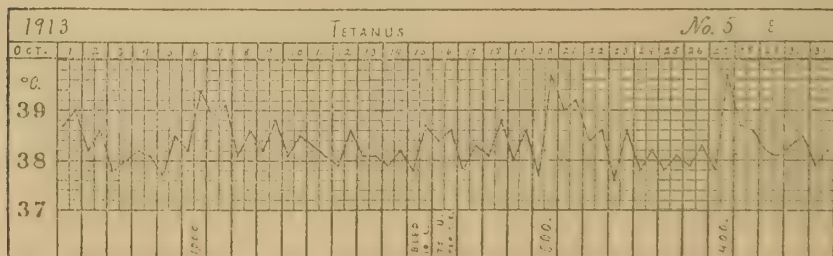FIG. 72. Temperature chart of horse 7 for September, 1914.
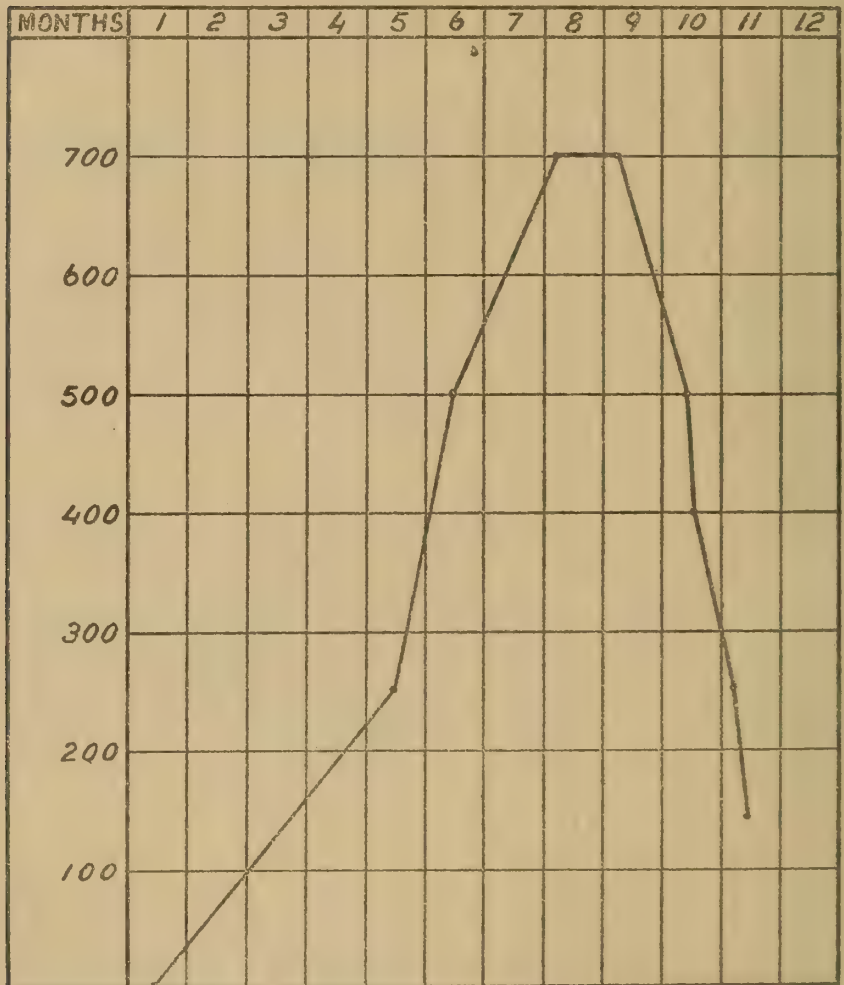
FIG. 73. Chart showing the antitoxin curve for horse 7. Units per cubic centimeter by months.

HORSE 8

The immunization of horse 8 against tetanus toxin was begun on November 10, 1913. This horse was fortified with 2,000

units of tetanus antitoxin, 1,000 units of which were given on November 10, 1913, and 1,000 units were given two weeks later, on November 24, 1913. The injection of tetanus toxin was begun on November 10, 1913; injections were given at intervals of a week, and the doses were increased gradually. Twelve liters of blood were withdrawn in the latter part of April, 1914, from which were obtained 5 liters of serum testing 250 units of tetanus antitoxin per cubic centimeter. After the bleeding the injections of tetanus toxin were continued. On June 9, 1914, the horse was bled 5 liters, which yielded 2 liters of serum that contained 250 units of tetanus antitoxin per cubic centimeter. After this bleeding large doses of toxin—750, 1,000, 1,250, 1,500, and 2,000 cubic centimeters—were injected. Ten liters of blood withdrawn on July 21, 1914, yielded 3 liters of serum with 300 units of tetanus antitoxin per cubic centimeter. Four liters of serum, which were obtained from 10 liters of blood withdrawn on July 24, 1914, tested 275 units per cubic centimeter. The injections of large quantities of toxin did not greatly increase the antitoxin content in the serum of the horse. Three injections of tetanus toxin were given—one dose of 500 cubic centimeters on July 27, 1914, one dose of 750 cubic centimeters on August 3, 1914, and one dose of 1,000 cubic centimeters on August 10, 1914. On August 18, 1914, the horse was bled 10 liters and 2.5 liters of serum were obtained, which tested 150 units per cubic centimeter. A bleeding of 10 liters on August 21, 1914, yielded 4 liters of serum with 100 units of antitoxin per cubic centimeter.

After the bleeding on August 21, 1914, toxin was given in 4 injections—500 cubic centimeters on August 24, 1914, 700 cubic centimeters on August 31, 1914, 800 cubic centimeters on September 7, 1914, and 1,000 cubic centimeters on September 14, 1914. On September 21, 1914, the horse was bled 10 liters and 3.5 liters of serum were obtained, which contained 75 units of tetanus antitoxin per cubic centimeter.

Ten liters of blood were withdrawn on September 23, 1914, which yielded 4 liters of serum with about 75 units of antitoxin per cubic centimeter. On September 25, 1914, horse 8 was bled to death; 14 liters of blood were obtained, which yielded 6 liters of serum that contained a little more than 50 units of antitoxin per cubic centimeter.

Horse 8 received in all 19,323.5 cubic centimeters of tetanus toxin. It furnished 91 liters of blood, which yielded 34 liters of antitetanic serum or 5,537,000 units of tetanus antitoxin. If

we deduct 50 per cent of the antitoxin as loss, we can count on having 2,768,500 units for the market (see figs. 74 to 85, charts 8–A to 8–L, for horse 8).
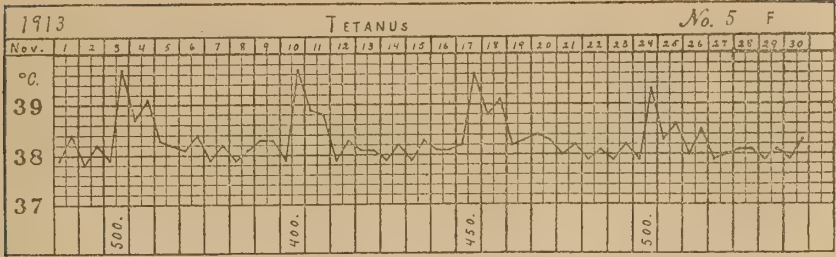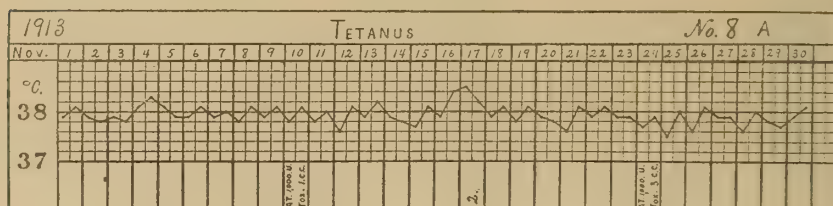

FIG. 74.　Temperature chart of horse 8 for November, 1913.
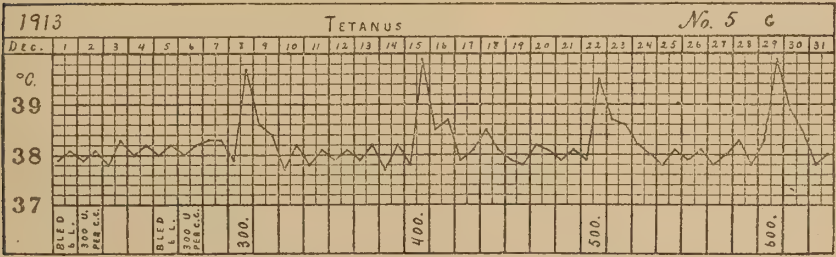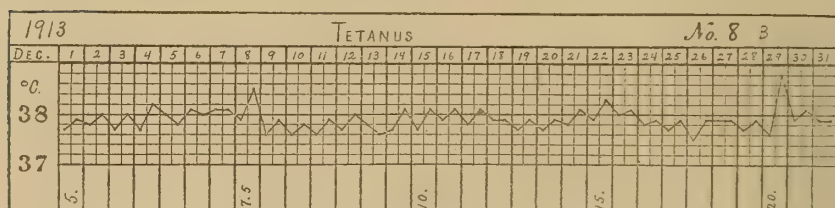

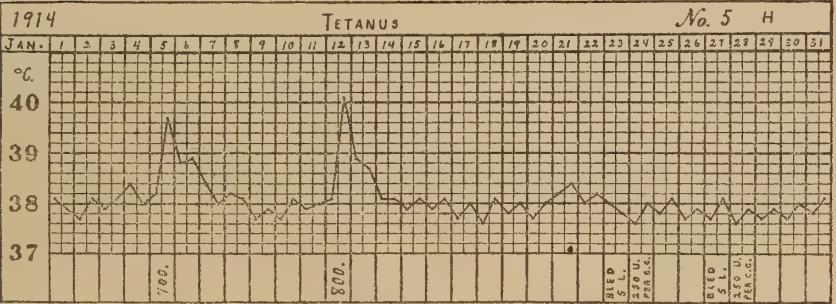FIG. 75.　Temperature chart of horse 8 for December, 1913.
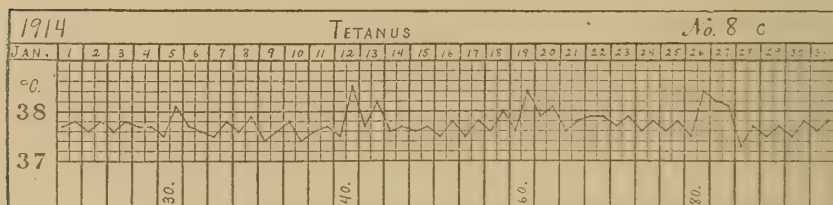
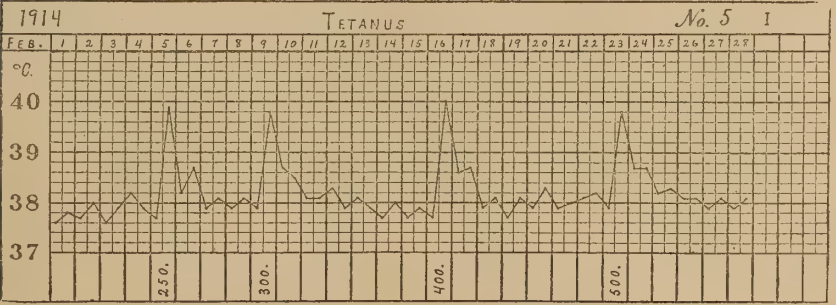FIG. 76.　Temperature chart of horse 8 for January, 1914.
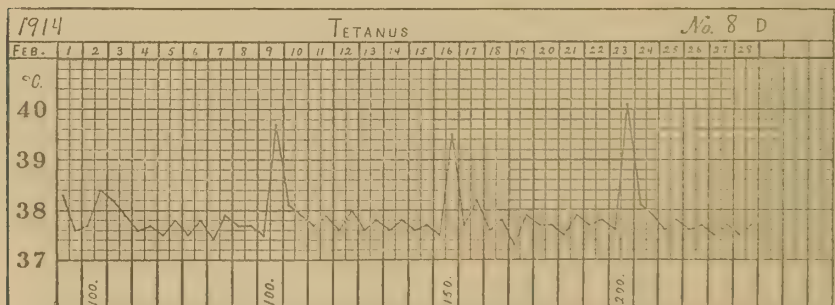
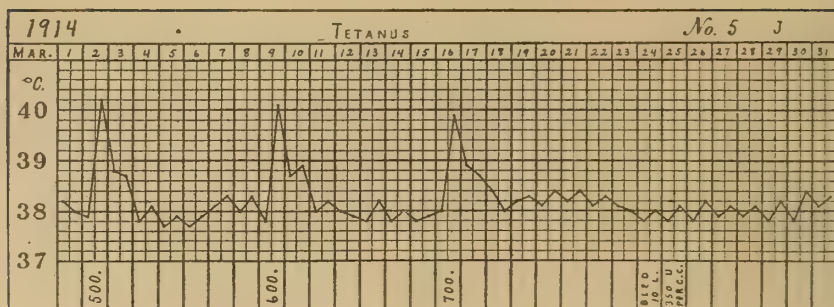FIG. 77.　Temperature chart of horse 8 for February, 1914.
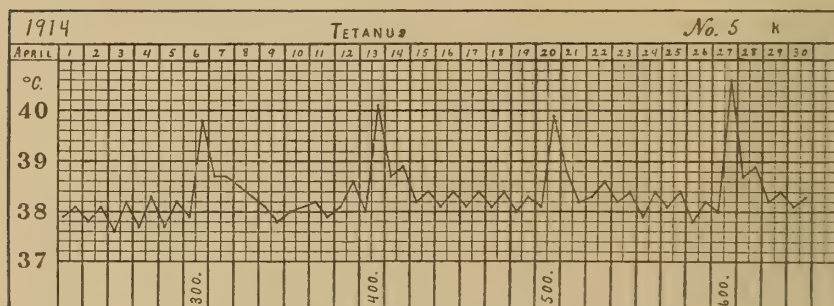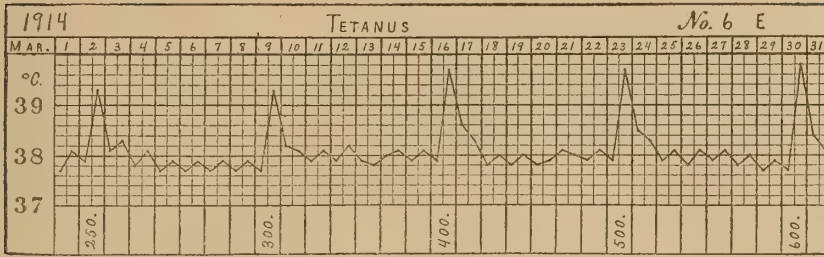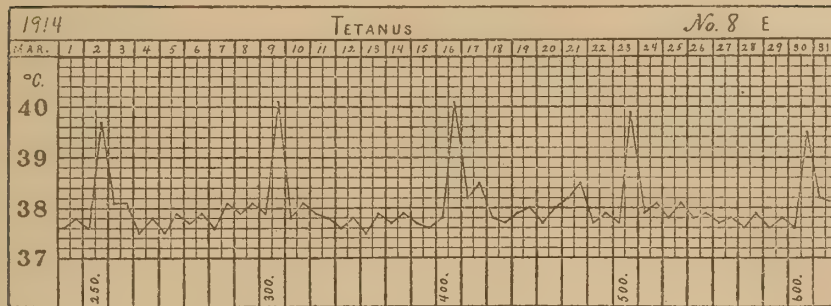
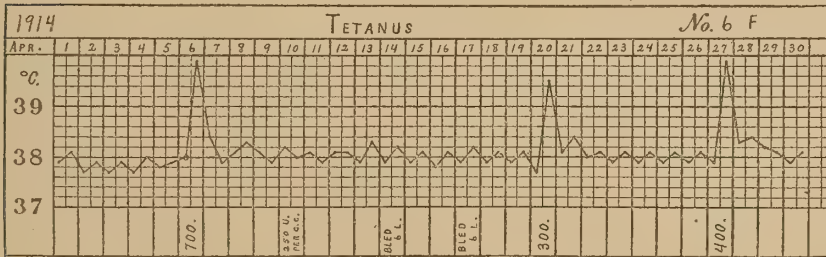FIG. 78. Temperature chart of horse 8 for March, 1914.
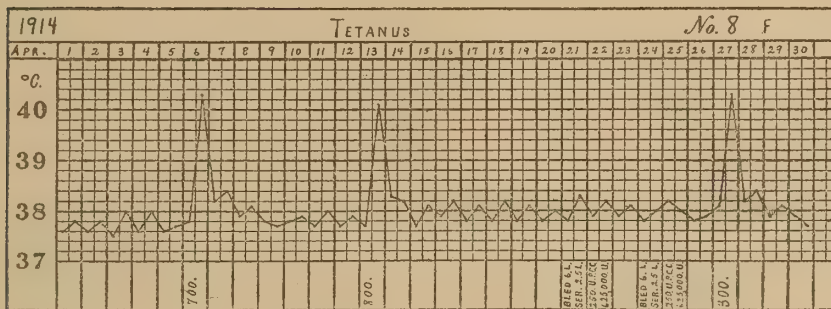
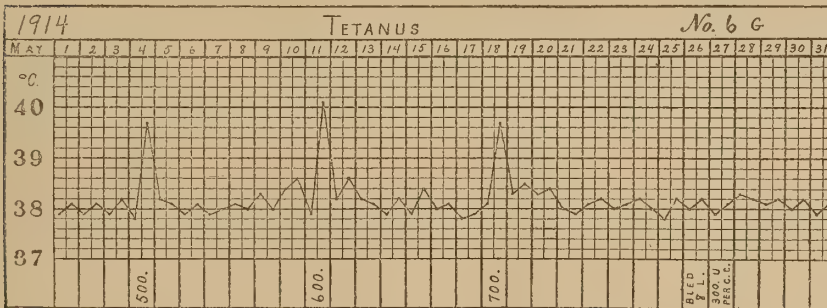FIG. 79. Temperature chart of horse 8 for April, 1914.
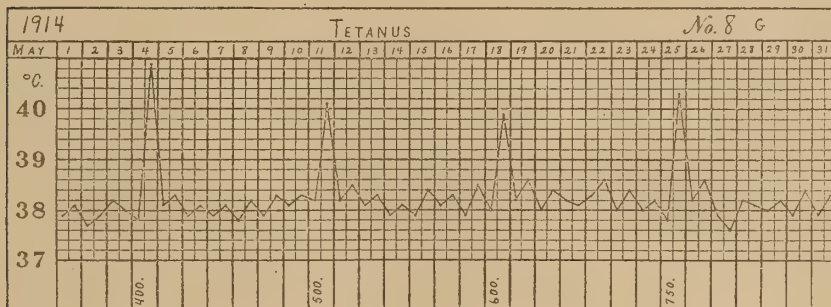
FIG. 80. Temperature chart of horse 8 for May, 1914.
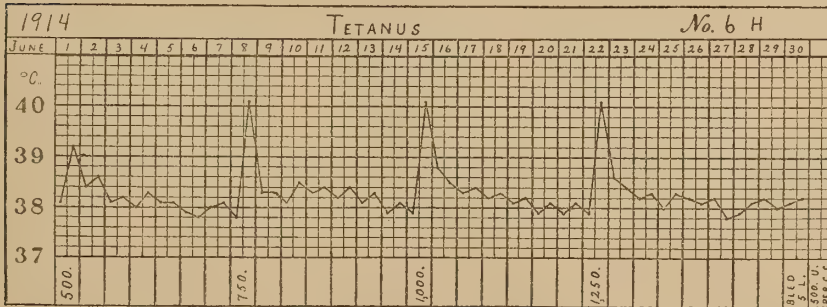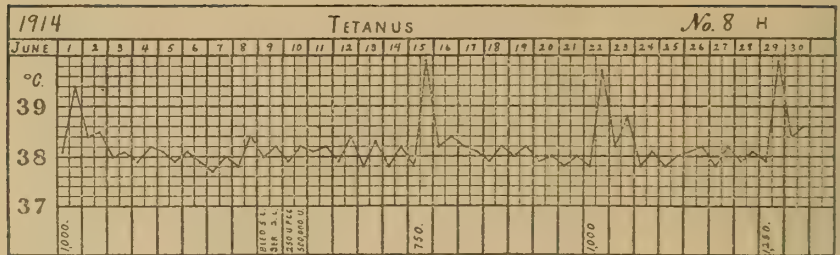
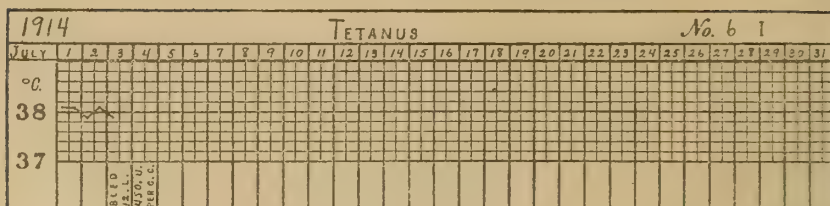FIG. 81.   Temperature chart of horse 8 for June, 1914.
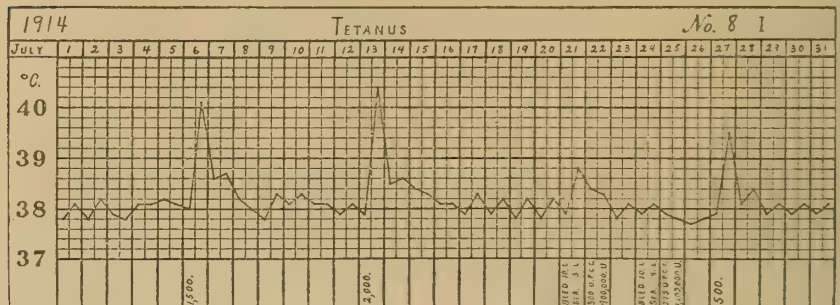
FIG. 82.   Temperature chart of horse 8 for July, 1914.
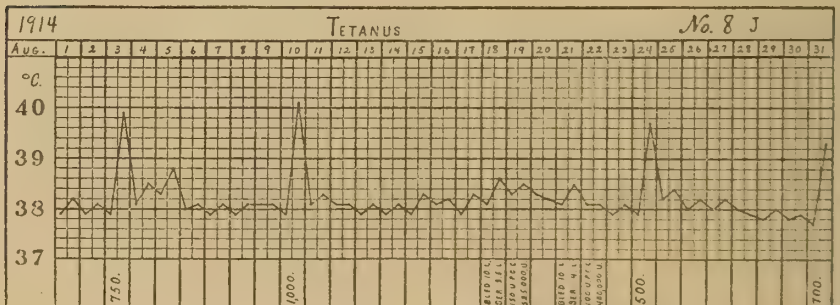
FIG. 83.   Temperature chart of horse 8 for August, 1914.
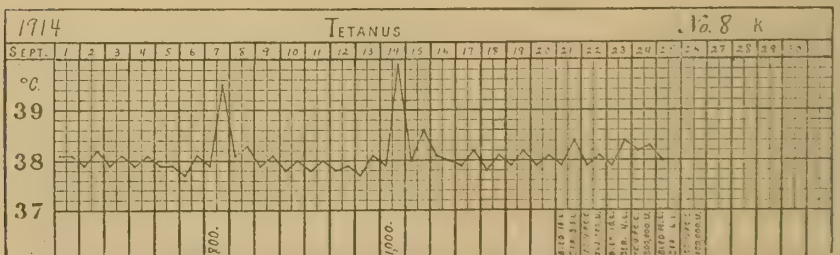
FIG. 84.   Temperature chart of horse 8 for September, 1914.
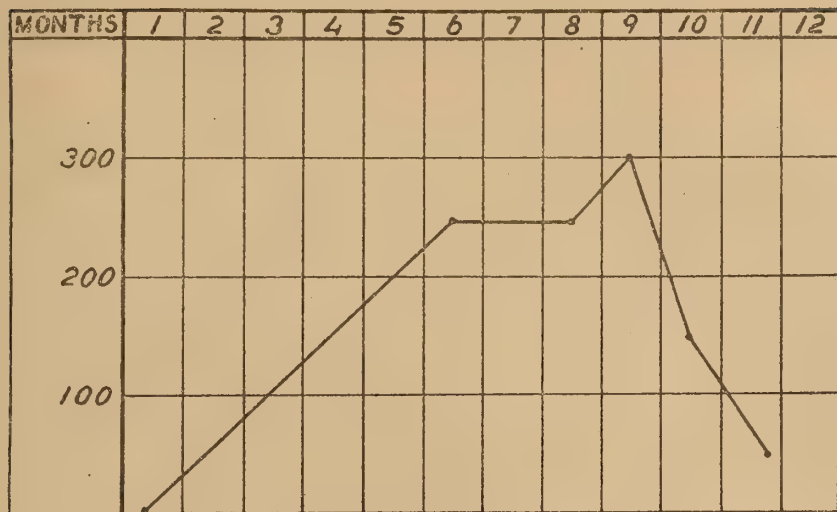
Fig. 85. Chart showing the antitoxin curve for horse 8. Units per cubic centimeter by months.

CONCLUSIONS

From the results obtained and reported above, the following conclusions seem justified:

1. A suitable strain of the bacillus of tetanus will usually produce potent toxin when grown in nearly neutral glucose broth under hydrogen. The acidity of the broth will rise to more than 2 per cent normal acid, and it should be neutralized with sodium hydrate before it is injected into the horse.

2. Potent tetanus toxin was obtained by the method described by Ivan Hall. By this method the acid is continuously neutralized by the magnesium carbonate present.

3. Horses differ greatly in the power of producing tetanus antitoxin. Of 8 horses reported on, one produced 150 units per cubic centimeter of serum, two produced 300 units per cubic centimeter of serum, one produced 350 units per cubic centimeter, and in the serum of one the antitoxin rose to 400 units per cubic centimeter. Three horses produced 500 or more units of tetanus antitoxin per cubic centimeter of serum.

4. The antitoxin curve reached its highest mark in from six to nine months after the beginning of immunization.

5. The injection of large doses of toxin is not indicated. The dosage should be such that the horse does not appreciably lose in weight.

·ILLUSTRATIONS

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CÆSAREAN SECTION IN THE PHILIPPINE ISLANDS [1]

By Fernando Calderon

(From the College of Medicine and Surgery, University of the Philippines, and the Philippine General Hospital)

At the last meeting of this society held in November, 1912, I had the honor to read an article entitled, Preliminary report of the first nine cases of abdominal Cæsarean section performed in the Philippines in the treatment of placenta prævia. Three of these 9 cases were performed in my clinics, while the rest belonged to other physicians. Besides these there appeared 5 other cases of Cæsarean section which were performed for the treatment of contracted pelvis and for eclampsia, so that at that time 14 cases of Cæsarean section had been performed in the Philippines with the following results: Mothers, 12 recovered and 2 died; babies, 8 living and 6 died.

In the preliminary report, the following conclusions were given:

1. Abdominal Cæsarean section, although a spectacular operation, is simple and should be more frequently resorted to in cases of placenta prævia.

2. Hysterotomy is a treatment which can best guarantee the life of both the mother and the child in those cases of placenta prævia in which the cervix is not dilated and the amniotic sac cannot be reached.

3. In infected cases Porro's Cæsarean section is to be performed rather than hysterotomy.

4. In cases where the cervix is widely dilated, the rupturing of the bag of waters or the perforation of the placenta, followed immediately by the extraction of the fœtus, is the operation of choice.

5. In those cases in which the cervical canal is not obliterated, but in which the examining finger is able to reach the amniotic sac, the latter should be ruptured, followed immediately by a tight vaginal pack, or by the introduction of a balloon to complete the dilatation of the cervix. This operation will do away with hysterotomy.

Two years have elapsed since my preliminary report was made, and the experience acquired during that time, thanks to the vast number of cases furnished by the department of obstetrics of the Philippine General Hospital, has shown that the

conclusions given in my preliminary report are confirmed by results; therefore they are entitled to receive the approval which they deserve.

During that period of time 17 cases of abdominal Cæsarean section were performed, giving the following results:

TABLE I.—*Results of Cæsarean section in cases in the Philippine Islands.*

	Number.	Per cent.
Operated for placenta prævia:		
Recovered	12	
Died	3	
Operated for eclampsia, recovered	1	
Operated for premature separation of placenta, died	1	
Total Cæsarean sections	17	
Total maternal deaths	4	23
Babies:		
Living	5	29
Died several hours after birth	7	41
Stillborn	5	29

The fact should not be forgotten that the majority of the patients coming to the hospital with placenta prævia are already exhausted by loss of blood and that they come to the hospital only as a last resort.

Of these 17 Cæsarean sections, 15 were performed in the department of obstetrics of the Philippine General Hospital, 1 in the San Juan de Dios Hospital, and 1 in the Casa de Salud of Drs. Quintos, Angeles, and Velarde. In one of these cases the operation was performed on a woman at the eighth month of pregnancy, who entered the Philippine General Hospital in a serious condition, being unconscious and having convulsive attacks every five minutes. The cervix was not dilated. Cæsarean section was performed, and both the mother and the fœtus were saved. In this particular instance, we can readily see that, besides placenta prævia, Cæsarean section also finds a wide field of usefulness in cases of eclampsia during pregnancy in which the cervical os is closed, as in these cases, instead of wasting our time dilating the cervix by means of balloons and other cervical dilators, a process which requires at least twelve hours to induce labor, we can solve the gravity of the problem in from thirty to forty minutes, if we resort to the abdominal Cæsarean section.

Another indication for abdominal Cæsarean section is given by those women who have contracted pelves and who always

deliver a stillborn fœtus. I know of some of these cases in Manila, and I hope that as the use of Cæsarean section is becoming more general it will also become the operation of choice for this kind of cases, especially if we take into consideration the fact that Cæsarean section, if performed at the right time and not as a last remedy, is entirely free from any danger and insures the safe recovery of the patient.

One thing which is of great importance in connection with Cæsarean section is the mode of procedure before and after the operation—that is, the preliminary treatment and the after-treatment. In the Philippine General Hospital, whenever the hæmorrhage has been profuse and the patient shows the symptoms of acute anæmia, our attention first is directed to improving the condition of the patient by administering stimulants, such as strychnin or camphorated oil, and by hypodermoclysis of normal salt solution, 500 cubic centimeters or more being injected under each breast. This procedure may be repeated again during the operation if necessary. After the operation, the patient is placed in Trendelenburg position, camphorated oil is given hypodermically, 1 cubic centimeter every hour or two, and protoclysis of normal salt solution given in drop method. This mode of treatment is usually all that is needed, but in more serious cases where the loss of blood has been very great hypodermoclysis may be repeated in addition. Of course, it is needless to say that different cases require different methods of treatment.

During the last two months of this year, two of the first patients operated by me came back for confinement. In both cases, delivery was effected normally without the least sign of any untoward complication that might be attributed to Cæsarean section.

CASE REPORT OF OBSTRUCTED LABOR AND CÆSAREAN SECTION [1]

By R. B. WOODWARD

(*Surgeon, United States Navy*)

The following history is of interest as emphasizing the danger of ventral fixation of the uterus in child-bearing women.

Mrs. C., multipara, a short, heavy-set woman, 32 years of age. She has had 2 previous labors, both instrumental; one child, delivered by high forceps in 1911, is living.

She had conceived in November, 1913, and came for examination in March, 1914, stating that she had been told that another pregnancy would kill her. Examination revealed a large rectocele and an extreme dilation of the hæmorrhoidal veins; the cervix, while high and slightly retroflexed, was in fair position. A large abdominal scar, due to a ventral fixation of the uterus in 1912, accounted for the high position of the cervix. The dangers of delivery at term were explained to the patient, who, however, being a devout Catholic, refused any interference at this time. Pregnancy proceeded normally, and September 4 was predicted as the date of beginning labor. The patient had false pains a week previous to this, and was much alarmed and worried over the onset of true labor. On the evening of September 8, at 10.45, the patient started on true labor; the os was dilated to admit 2 fingers, and pains came at eight-minute intervals, lasting from one to two minutes. Progress in dilatation was steady but slow, and the presentation was an O. D. P. At 5 o'clock in the morning the os would admit 3 fingers, and the fixation of the uterus would not permit the cervix to descend or the head properly to engage. Pressure on the fundus was maintained, and under primary anæsthesia digital dilatation of the cervix was attempted with no result. At 7.30 in the morning, under ether anæthesia, high forceps was tried and an honest effort lasting fifty-five minutes was made to induce progress. None was made, and under the same anæsthesia the patient was conveyed to the hospital and a Sanger

[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

operation performed; a living male child weighing 9.5 pounds was delivered. The wound in the uterus and belly wall was repaired. The child lived seventeen hours, dying in convulsions as a result of head injury from forceps. The mother made an uneventful recovery, leaving the hospital on the twelfth day.

A plea is here entered to those having in mind operations on the female genital organs for conservatism and proper consideration of the potential mother.

The ventrofixation method has given the following statistics. DeLee [2] says:

> Ventrofixation and ventrosuspension and adhesion of the uterus to the abdominal wall following celiotomy not seldom cause serious dystocia. Lindfors, in 68 cases of labor found that the cervix was high in 27; transverse presentation occurred in 31; and trouble in the third stage in 10. There were 30 versions, 13 Cæsarean sections, 10 high forceps, 5 embryotomies, 5 tamponades for post-partum hæmorrhage, and many other operations necessary. There were 3 deaths.

Andrew's collection of 395 cases adds 36 cases of Cæsarean section.

That frequency of abortion, difficult labor, post-partum hæmorrhage, and the necessity for capital operations to overcome obstruction should forbid the practice of ventral fixation in childbearing women is the opinion of DeLee, Cragin, and Polak.

The Alexander and allied operations on the ligaments—especially the Andrew's (with ligament sewn behind the uterus) — give little real trouble, and with a proper repair of the vaginal floor will answer every and all purposes.

[2] Principles and practice of obstetrics. Philadelphia, W. B. Saunders Company (1914), 402.

ADENOCARCINOMA OF THE CÆCUM, COMPLICATED BY INTUSSUSCEPTION [1]

By Robert M. Thornburgh

(*Major, Medical Corps, United States Army, and Chief of Surgical Service, Department Hospital, Manila, P. I.*)

On January 29, 1914, Sergeant A. S., aged 35, was admitted to the Department Hospital at Manila. He came from Camp Stotsenburg, Pampanga Province, with a transfer diagnosis of "dysentery, chronic entamœbic, recurrent." He has had three years of tropical service. There was no history of cancer in his family.

Previous history.—Malaria in 1906, good recovery; seven admissions to hospital for "intestinal trouble" during the past year; drinks moderately, smokes moderately, and chews tobacco occasionally; had gonorrhœa in 1906, good recovery; denies syphilis (Wassermann —).

Present illness.—Onset in May, 1912 (?). Severe colicky pain in lower abdomen; frequent watery stools, rectal tenesmus, and cramps in thighs; mucus present, no blood observed. He recovered from this attack, but from that time on was troubled with exacerbations of above symptoms and signs whenever he was indiscreet in diet. He is always prostrated and has some fever; mucus and sometimes blood in stools. This has required one hundred fifteen days in hospital since May, 1912.

On admission.—Intense colicky pain in lower abdomen; tenesmus of colon and rectum; frequent watery stools containing mucus in abundance, very fetid; much flatulence; nausea and vomiting; cramps in thighs.

The patient is well developed, fairly well nourished, facies anxious, breath fetid. There is tenderness over colon, especially in cæcal area; colon distended and hypertympanitic; borborygmus is marked. Stools contain much mucus, no blood, no ova of intestinal parasites, and no protozoa. The heart and lungs are negative; arteries, thickened and hardened somewhat.

[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

The patient was put on diluted milk diet and improved steadily; on February 13 he was tried on a baked potato. About four hours later he was taken with intense colicky pain in the lower abdomen, accompanied by much distention and prostration. Local measures, stupes, and enemeta gave relief, but the patient grew rapidly worse until an exploratory laparotomy was decided upon and done on February 17. (I might add here that there were 3 complete blood counts made, all of which were normal, 90 per cent hæmoglobin.)

Upon opening the abdomen through the right rectus, a mass of about the size of a coconut was found filling the entire right lower quadrant. The head of the cæcum was invaginated, carrying the normal appendix with it. The cæcum was enormously thickened and formed a ball-valve, completely occluding the ileocæcal valve. The condition was so manifestly malignant that immediate resection was decided upon. The cæcum was amputated and with it 10 centimeters of ascending colon and 5 centimeters of ileum. A lateral anastomosis by the Moynihan method was made high up on the ascending colon. The wound was closed without drainage.

The patient made an uninterrupted convalescence and was sent to the Letterman General Hospital, San Francisco, on March 15, 1914. He weighed at that time 114 pounds. I received a letter from him a few weeks ago, written six months after the operation, in which he stated that he was in the best of health, weighed 163 pounds, and had returned to duty. Another letter received March 27, 1915, stated he was perfectly well and was on duty at Fort Meade, South Dakota.

TWO COMPOUNDS OF EMETINE WHICH MAY BE OF SERVICE IN THE TREATMENT OF ENTAMŒBIASIS [1]

By A. G. DuMez

(*From the School of Pharmacy, College of Medicine and Surgery, University of the Philippines*)

INTRODUCTORY

While emetine, per se, has become recognized as a valuable remedy in the treatment of entamœbiasis only within the past few years, ipecac root, from which emetine is obtained, has long been employed in the treatment of this disease by medical practitioners.

The available literature on the subject conveys the information that the drug was first brought to the notice of Europeans in 1590 under the name of "igpecaya" or "pigaya" by a Portuguese friar, who obtained it in Brazil.[2] We are further informed, however, that it was not effectively introduced to European medicine until a century later, 1686, when Jean Adrien Helvetius [3] attained fame through its use in the treatment of dysentery. Several times since then it has fallen into disuse in the management of this disease, but has as often been revived, and at present its efficacy may be said to be firmly established. However, in spite of its established value, there is a marked disadvantage in its use which tends to diminish its popularity—that is, its emetic action. Through the combined efforts of the chemist and pharmacologist, we are now able to state that this action is due to two of its constituents: namely, emetine and cephaëline.

The chemistry and pharmacology of ipecac root have been pretty thoroughly worked out with respect to the constituents which might be expected to be physiologically active. The ipecacuanhic acid of Willigk [4] was shown by Kimura [5] to be neither astringent nor antiseptic. The presence of 5 different alkaloids

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[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

[2] Purchas, His Pilgrimes. London (1625), 4, 1311.

[3] Wootton, Chronicles of Pharmacy. Macmillan & Co., London (1910), 2, 114.

[4] Ipecacuanhic acid was first isolated by Willigk in 1850. *Journ. f. prakt. Chem.* (1850), 51, 424.

[5] *Arch. internat. de Pharm. et Tox.* (1903), 405.

has been reported: namely, emetine; cephaëline; psychotrine; ipecamine, isomeric with psychotrine; and hydroipecamine, isomeric with caphaëline.[6]   The pharmacology of emetine and cephaëline was worked out by Wild,[7] who showed that the latter was the more energetic in its action as an emetic.   All of the above work, with the exception of the isolation of the two isomeric bases, was published prior to 1896, by which time the ipecac treatment had become generally recognized.   It appears, however, that no attempt was made to use the pure alkaloidal constituents of the drug in the treatment of dysentery until 1912, when Rogers [8] reported the results obtained in 25 cases of entamœbiasis treated with emetine hydrochloride given subcutaneously.   Although the results obtained by Rogers were the first direct clinical evidence of the value of emetine as a remedial agent in the treatment of entamœbiasis, we are indebted to Vedder for pointing out its probable use in this connection.

In 1911 Vedder [9] published an article on the effect of ipecac, deëmetinized ipecac, and emetine on the entamœbæ in vitro in which he pointed out the toxic action of emetine with respect to this organism.   Rogers then took up the work and tested the effects of emetine on the entamœbæ found in dysenteric stools. He noted that entamœbæ in them were killed by a dilution of 1 to 10,000 of emetine hydrochloride and rendered inactive even by so high a dilution as 1 to 100,000.   A microscopic examination of the dysenteric ulcers of an advanced case which ended fatally failed to reveal a single living amœba after a dosage of 0.22 gram of emetine hydrochloride in two and a half days.   He was so impressed with these results that he began the administration of emetine hydrochloride in a number of cases of entamœbiasis.

At first Rogers tried the administration of emetine hydrochloride in the form of a salol-coated pill, but obtained comparatively poor results owing, undoubtedly, to its rapid elimination.

[6] Emetine was obtained by Pelletier and Magendie in 1817.   *Ann. Chim. et Phys.* (1817), **4**, 172–185.   Cephaëline and psychotrine were isolated by Paul and Cownley, the former in 1895, the latter a few years later. *Pharm. Journ.* (1895), **25**, 111, and *Am. Journ. Pharm.* (1901), **73**, 87. Just recently Hesser has reported the presence of ipecamine and hydroipecamine.   *Drug. Circ.* (Oct., 1914), 613.

[7] *Lancet* (1895), **2**, 1274.

[8] *Therap. Gaz.* (1912), **36**, 837–842.

[9] Vedder showed that deëmetinized ipecac was without harmful effect upon the entamœbæ, but that the destructive properties of ipecac were greater than its emetine content would indicate.   From which we may infer that cephaëline is also toxic to the entamœbæ.   *Bull. Manila Med. Soc.* (1911), **3**, 48.

However, the results which he obtained upon giving it hypodermically more than repaid him for his trouble. He advocates giving it in normal salt solution in doses of 0.03 to 0.04 gram (representing from about 2.6 to 4 grams of ipecac) to adults and 0.02 gram to children of about 8 years of age. He reports that on several occasions as much as 0.06 gram was administered two or three times a day without the development of any untoward symptoms, and that, even in the fullest doses, the drug never produced sickness and rarely any nausea. The comparative results obtained by Rogers with the old ipecac treatment and the new emetine are shown in Table I.

TABLE I.—*Showing the results obtained in cases of dysentery treated with ipecac and with emetine hydrochloride.*

Treatment.	Died.			Discharged.			Total cases.
	Within 3 days.	After 3 days.	Of other diseases.	Very bad.	Not cured.	Cured.	
Ipecac	4	7		2	4	13	30
Emetine hydrochloride	2		2			21	25

The observation of Rogers have been corroborated in a measure by Baermann[10] and others. Baermann found the drug most efficacious if the first 1 or 2 doses were given intravenously in quantities of from 150 to 200 milligrams (the maximum dose being 250 milligrams per 60 kilograms of body weight), the intravenous injections being followed by 4 or 5 subcutaneous injections of from 100 to 200 milligrams at two- or three-day intervals, and the latter treatment again repeated at intervals of three or four weeks.

Notwithstanding the fact that emetine hydrochloride, as a general rule, has yielded good results in the treatment of entamœbiasis in comparison with those obtained through the use of ipecac, there still appears to be an opportunity for improvement in the present form of treatment. Very often negative results are obtained, even with the administration of emetine. Whether this is due to an impurity[11] in the alkaloid as obtained on the market or whether it is due to our inability to bring the remedy in contact with the entamœbæ in the proper concentration for a

[10] *München. Med. Wochenschr.* (1913), 50, 1132.

[11] Five different commercial samples of emetine hydrochloride examined in this laboratory showed the presence of cephaëline when tested with Frœhde's reagent. As cephaëline has been shown to be much more irritating than emetine, this condition of the commercial salt may be significant.

sufficient length of time,[12] I am unable to state. Painful inflammation occasionally results at the point of injection. Furthermore the present form of treatment is too complicated to permit of emetine being used as a household remedy or as a prophylactic.[13] It is for the purpose of simplifying the treatment, and increasing its efficiency, if that be possible, that I bring to your notice the compounds emetine mercuric iodide and emetine bismuthous iodide.

EMETINE MERCURIC IODIDE

Emetine mercuric iodide was prepared by precipitating an acidified aqueous solution of emetine hydrochloride[14] with Mayer's reagent,[15] collecting and washing the precipitate with water, and drying in the air at a temperature below 50° C. The samples used for analysis were dried over sulphuric acid. The composition of the resulting precipitate was found to vary with the concentration of the solution. Table II shows the approximate composition of the compound obtained on precipitation in the dilution 1 to 300.[16]

[12] Wherry found that emetine killed the entamœbæ in dilutions of from 1: 20,000 to 1: 100,000 only after 24 hours at a temperature of from 36° to 38° C. *Journ. Infect. Dis.* (1912), **10**, 162–5.

[13] Willets has pointed out the fact that entamœbic dysentery in an infected individual can be prevented and that prophylaxis against carriers of *Entamœba histolytica* can be obtained by expelling the pathogenic entamœbæ from the intestines of infected persons. He found ipecac more efficacious than emetine for this purpose. *This Journal, Sec. B* (1914), **9**, 94.

[14] The emetine hydrochloride obtained from Merck and Co. and labeled "Nach Paul" was found to contain cephaëline. The cephaëline was separated according to the method of Paul and Cownley, and the purified emetine hydrochloride was used in the above preparation.

[15] Mayer's reagent was prepared by dissolving 1.344 grams of mercuric chloride and 5 grams of potassium iodide in 100 grams of distilled water. U. S. P. 8th rev. (1905), 529.

[16] No direct estimation of the alkaloidal content of the precipitate was made, the analyses being conducted as follows: A weighed quantity of the precipitate dried over sulphuric acid was dissolved in hot alcohol acidulated with sulphuric acid. Silver nitrate solution was then added to precipitate the iodine; the precipitate was washed on the filter with hot water, dried at 100° C., and weighed. The filtrate was treated with a slight excess of hydrochloric acid, and the silver chloride was filtered out, after which hydrogen sulphide was passed in and the resulting mercury sulphide collected, washed, dried at 100° C., and weighed. After having found the iodine and mercury, the fraction of hydrogen for the HI of the assumed formula was added, and the difference was taken as alkaloid.

TABLE II.—*The composition of emetine mercuric iodide.*

Constituent.	Calculated for $(C_{30}H_{40}N_2O_5)$ $(10HI)$ $(HgI_2)_3$.	Found.		Mean.
		1	2	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Iodine	2,014.40	43.43	43.13	43.03	43.08
Mercury	595.50	12.84	12.53	12.48	12.50
H of HI	10.00	0.23	....	....	0.23
Emetine	2,018.24	43.50	....	....	44.19
	4,638.14	100.00			100.00

Emetine mercuric iodide is a light-yellow powder without odor or taste; it is practically insoluble in water or water acidulated with hydrochloric acid. It is apparently quite stable with respect to ordinary physical conditions, as a specimen kept in a cork-stoppered bottle for more than four months has undergone no noticeable change.

EMETINE BISMUTHOUS IODIDE

The procedure in the preparation of emetine bismuthous iodide was similar in all respects to that followed in the preparation of emetine mercuric iodide, except that Dragendorff's [17] instead of Mayer's reagent was employed in forming the precipitate. Here, also, the composition of the precipitate was found to vary with the concentration of the solution. The following analytical results [18] were obtained for the precipitate formed in the dilution of 1 to 300.

Emetine bismuthous iodide is a brick-red powder, insoluble in water or water acidulated with hydrochloric acid. It is quite stable in the air.

[17] Dragendorff's reagent was prepared by saturating a hot concentrated solution of potassium iodide with bismuthous iodide (BiI_3), and then diluting with an equal volume of a saturated solution of potassium iodide. *Zeitschr. f. anal. Chem.* (1866), **5**, 407.

[18] The analysis of the bismuth compound was carried out in a manner very similar to that of the mercury precipitate, except that the solution was at all times kept rather strongly acid to prevent the formation of basic compounds. The filtrate after the removal of the silver as chloride was concentrated by evaporation on a water bath and then diluted with water containing acetic acid, after which the bismuth was precipitated as sulphide, washed with water containing H_2S, dried at 100° C., and weighed. The lowest of several weighings taken at intervals of a half-hour was the figure used in the computations.

TABLE III.—*The composition of emetine bismuthous iodide.*

Constituent.	Calculated for (C₃₀H₄₀N₂O₅) (5HI) (BiI₃).	Found.		Mean.
		1	2	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Iodine	1,007.20	58.43	58.30	58.21	58.26
Bismuth	206.90	12.00	12.52	12.20	12.36
H of HI	5.00	0.30	----------	----------	0.30
Emetine	504.56	29.27	----------	----------	29.08
	1,723.66	100.00			100.00

PHARMACOLOGICAL AND THERAPEUTIC ACTION OF THE MERCURY AND BISMUTH COMPOUNDS

With the object of obtaining some information concerning the value of the mercury and bismuth compounds in the treatment of entamœbiasis, samples of the two salts were sent to the Philippine General Hospital some time ago. However, owing to the rapid change in personnel taking place in the hospital at the time, the salts were misplaced and, therefore, I can bring before you no clinical evidence with respect to their usefulness. Theoretically the action, in part, should be similar to that of emetine hydrochloride, as emetine will undoubtedly be one of the compounds liberated in the intestines.

Emetine mercuric iodide should be decomposed in the stomach to a slight extent only. Upon coming in contact with the alkaline secretions of the intestines, the compound will very likely be decomposed with the formation of emetine; finally, as the free base and a salt of mercury which will eventually be converted in part into the albuminate, in which condition, Cushny [19] states, it enters the circulation. Therefore we should expect the combined action of both emetine and the mercury compound upon the entamœbæ.

Insoluble salts of bismuth are converted into bismuth sulphide after passing the pyloris.[20] In all probabilities the reaction is very slow and takes place as the compound is being spread out over the walls of the intestines. The value of bismuth in the treatment of entamœbiasis has been demonstrated by Deeks.[21] In addition, in this case, emetine will probably be liberated

[19] Cushny, Pharm. and Therap. Lea and Febiger, Philadelphia (1911), 641.
[20] Bastedo, Mat. Med. and Therap. W. B. Saunders Co., Philadelphia (1914), 494.
[21] *Journ. Am. Med. Assoc.* (1913), 61, 385.

slowly at the very seat of the trouble. The comparatively poor results obtained from the administration of emetine by mouth is very probably due to its too rapid absorption and elimination, as has already been pointed out.

These two compounds can be given in doses representing 0.03 gram of emetine hydroiodide without causing vomiting and without any apparent nausea; but vomiting does occur when either of them is given in very large doses, as is shown in the following tabulation of experiments conducted with dogs:

TABLE IV.—*The effects of emetine mercuric iodide and of emetine bismuthous iodide upon dogs.*

Substance.	Weight of dog.	Dose.	Time given.	Vomiting.
	Kilos.	*Gram.*		
Emetine mercuric iodide ..... ----------	8.7	0.057	10.25 a. m.	None at 2 p. m.
Do---------------------------------	8.8	0.092	10.33 a. m.	At 11.26 a. m.
Emetine bismuthous iodide...............	7.8	0.066	10.38 a. m.	None at 2 p. m.
Do--------------------------------	6.3	0.075	3.22 p. m.	None at 5.30 p. m.
Do--------------------------------	9.8	0.012	10.40 a. m.	At 11.50 a. m.

In these two compounds, then, we may possibly find a remedy which can be administered frequently, in fairly large doses, and with a minimum amount of trouble, and which will bring emetine in contact with the entamœbæ for a comparatively longer time than is true with the forms in which it is administered at present.

THE TREATMENT OF INFANTILE BERIBERI WITH THE EXTRACT OF TIQUI-TIQUI [1]

By José Albert

(From the College of Medicine and Surgery, University of the Philippines)

Infantile beriberi is without doubt the most interesting problem of pediatrics and of general medicine in the Philippine Islands. It is responsible for that phenomenon, without parallel in the whole civilized world, in which infant mortality among breast-fed children is greater than the mortality among the artificially fed, thus giving the impression that human milk as a food is a scourge among infants, rather than the "magic liquor" which guarantees their lives. Because of its ravages among children under one year of age, 38 per cent of which are victims of this disease, infantile beriberi constitutes the most formidable barrier to both economical and political aggrandizement of these Islands.

Since this disease was discovered, all efforts of therapeutics have been to suppress the cause, following the well-known maxim of healing pathogenically. Believing that this disease was a true intoxication, Professor Hirota of Japan has recommended the discontinuance of maternal feeding, which according to him is the "materia peccans," and the substitution of artificial feedings. In the treatment of this disease Hirota has established the two following principles:

First, that infantile beriberi is a true intoxication produced by the ingestion of beriberic milk.

Second, that the treatment of the disease by means of drugs is of little or no value at all. That the discontinuance of maternal feeding is the only and safe means of cure, provided that the disease is not too far advanced in which case it is no longer of any value.

According to the same author the improvement which follows the discontinuance of maternal feeding is a rapid one, occurring on the third day. The complete cure with the exception of the aphonia, which lasts for several weeks, takes place in the majority of cases during the first week.

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[1] Read at the annual meeting of the Philippine Islands Medical Association, Manila, November 4–7, 1914.

Simple and ideal as this method of treatment looks, when put in practice it offers very serious inconveniences inherent in the sudden and untimely discontinuance of maternal feeding. To put the baby to a wet nurse is a wise procedure, but this offers a great expense which cannot be afforded by this kind of patients, for the great majority of them belong to the poorer class. To substitute artificial feeding for breast feeding is to expose the infant to the dangers of gastrointestinal and other nutritional disturbances which usually accompany artificial feeding. In addition, there is the difficulty or the impossibility of obtaining fresh milk or canned milk and other farinaceous food preparations because of pecuniary reasons as already mentioned.

In view of these inconveniences and believing that this disease is due to some deficiency in diet, Bréaudat of Indo-China in 1910 and Gabriel and Luis Guerrero of these Islands in 1911 fed the mothers of beriberic infants with rice polishings (tiqui-tiqui) and mongo—two well-known antiberiberic foodstuffs. By this method the mother is given daily about 60 grams of tiqui-tiqui and about 150 grams of mongo, prepared in different palatable ways so that its ingestion is made agreeable.

Only 18 cases have been treated by this method in the Philippine Islands. The method has two inconveniences: First, the tiqui-tiqui is very unpalatable and disagreeable to take, and, secondly, breast feeding has to be discontinued for a period of from fifteen to forty-five days according to the method of Luis Guerrero. Because of these inconveniences Bréaudat's method did not become widespread.

Following the theory of "avitaminosis" and drawing their conclusions from the prophylactic and curative actions of the extract of tiqui-tiqui on "polyneuritis gallinarum," Chamberlain and Vedder, of the United States Army board for the study of tropical diseases, in February, 1912, recommended the use of the extract in the treatment of infantile beriberi without the discontinuance of the maternal feeding, thus avoiding the dangers of artificial feeding. The method of preparation of this extract is fully described in their paper.[2] Five cubic centimeters of the extract thus prepared represent about 82 grams of rice polishings. The dose prescribed by them was 5 cubic centimeters of the extract a day given in 20-drop doses every two hours while the child is awake.

I have had the opportunity of using this extract in a great many cases of infantile beriberi, from the year 1912 to the

[2] *Bull. Manila Med. Soc.* (1912), 4, 26.

present date, and I believe that this medicine is of immense value in the treatment of the disease in question and that it is excelled by no other drug known at the present time. My distinguished colleagues of this city, Doctors Calderon, Quintos, Luis and Manuel Guerrero, Valdes, Gabriel, and others, are of the same opinion.

Because of these astonishing results, both the committee for the investigation of infant mortality and the Segunda Asamblea Regional de Medicos y Farmaceuticos have requested the Philippine Legislature to adopt measures so that this extract may be distributed freely to the poorer class. As a result of this request the Philippine Legislature in February, 1914, passed Bill No. 2376 providing the sum of 6,000 pesos [3] for the preparation and free distribution of the extract of tiqui-tiqui. The bill also provides that the Liga Nacional Filipina para la Proteccion de la Infancia will superintend the preparation and experimentation, will look after the free distribution of the extract among the poorer class, and will present a written report of the results of their experiments, through the Secretary of Public Instruction, at the beginning of each regular session.

Although the time that has elapsed since the first experiments were made is not long enough to permit us to draw definite conclusions, our opinion with regard to this extract is very favorable. The administration of the extract when given in time and in convenient doses is followed at once by marked improvement. At the end of twenty-four hours the vomiting, whining, restlessness, insomnia, dysphagia, polypnœa, and oliguria all disappear as if by magic. To the great surprise of the young physician the clinical syndromes change in aspect in a very short time, and at the end of three days one can say that the disease has disappeared, at least externally, leaving the aphonia which in its turn disappears by the end of the seventh or eighth week. (I have attended a case in which the aphonia lasted for about eight months.) If no improvement follows after twenty-four hours or if the case under treatment is of great severity, I obtain the desired effect by doubling the dose. It is better to give larger than smaller doses. The extract is inoffensive and entirely uninjurious, save for a slight diarrhœa which follows its administration. With the method of giving large doses to acute cases, we have in practice saved from sure death many cases of beriberi of the pernicious larval type, which formerly were believed to be hopeless and incurable.

[3] One peso Philippine currency equals 50 cents United States currency.

The administration of the extract must be continued as long as the aphonia persists. So long as the voice has not recovered its normal pitch, the infant is constantly threatened by an acute attack which may terminate in death in a few hours. In cases of infantile beriberi without aphonia, it is wise to prolong the treatment at least three weeks after apparent cure, to avoid relapses which frequently occur. It is necessary to impress upon the mother the importance of prolonging the treatment, as it is the tendency, in private practice, to discontinue treatment as soon as the acute symptoms disappear.

The failure of the extract to effect a cure may be due to many causes:

1. Extreme severity of the case—a very advanced neuritis. Vedder and Clark, in their work on polyneuritis gallinarum, have shown that symptoms appear only when the anatomic lesions in the nerves are well advanced. It is the same with infantile beriberi. It is not infrequent that mothers bring their children when the disease is far advanced. Therefore it is necessary to give the extract early in the disease in order that the treatment be successful.

2. The extract proves ineffective also in cases associated with other infections, commonly pneumococcic in the form of bronchopneumonia, which is a frequent complication of infantile beriberi.

3. Another cause of failure of the extract is its poor quality. It has either an insufficient quantity of the active principle or an excess of alcohol which makes its ingestion disagreeable and injurious. Analyses made by the Bureau of Science of the extracts prepared by the local drug stores revealed the fact that all the samples with the exception of one from one drug store were very deficient in the active principle. This was why treatment with extracts of tiqui-tiqui obtained from this drug store were the most successful.

The interpretation of the curative action of the extract of tiqui-tiqui is not a hard task. Hirota's theory of intoxication can be discarded. It does not explain why the beriberic child treated with tiqui-tiqui extract improves and gets well even without discontinuing the maternal nursing. The extract has no antitoxic properties and, therefore, it cannot be said that it neutralizes the toxic action of the breast milk.

Knowing the prophylactic and curative actions of the extract in polyneuritis gallinarum, which is caused by a deficiency in the diet, we must admit that this extract supplies the beriberic

infant with nutritive elements, probably the vitamines, in which the beriberic milk is found deficient.

According to Funk these vitamines play an important rôle in the metabolism as do the hormones, ferments, and internal secretions.

Bearing in mind that infantile beriberi under a clinical aspect is principally a vagotonia—an abnormal irritability of the vagus—it is logical to believe that the curative action of the extract is due to the fact that it supplies the vagus with the necessary vitamines for the normal performance of its functions. Using the words of Eppinger and Hess, we can say that the extract of tiqui-tiqui is a vagotropic drug, like pilocarpin, which has a selective and specific action on the vagus nerve.

By supplying the vagus with its much-needed vitamines, its abnormal irritability, manifested by vomiting, by angina pectoris (whining and restlessness), and by polypnœa because of its bronchial and pulmonary terminations, entirely disappears.

The failure of the extract to cure the aphonia can be attributed to a very advanced degenerative neuritis of the recurrent branches of the vagus nerve, which are the first to be affected in all cases of dietetic deficiency as evidenced by the clinical fact that the aphonic form constitutes from 80 to 90 per cent of all cases of infantile beriberi.

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PHILIPPINE ISLANDS MEDICAL ASSOCIATION

OPENING SESSION, NOVEMBER 4, 4.15 P. M.

The opening session of the eleventh annual meeting of the Philippine Islands Medical Association was called to order by President N. M. Saleeby at 4.30 p. m. on Wednesday, November 4, 1914, in the assembly hall of the University Hall, University of the Philippines, Manila, P. I.

The addresses as abstracted by the secretary-treasurer follow:

OPENING ADDRESS—BY HIS EXCELLENCY GOVERNOR-GENERAL FRANCIS BURTON HARRISON

Governor-General Harrison assured the association, on behalf of the Government, of the interest in the deliberations of this session for the Bureau of Health, for the Secretary of the Interior, and for himself, and complimented the association on the work which it had accomplished in the past and on the high standard of the present program. It is to the good offices of such associations that the community must look in order to secure mutual understanding and helpful coöperation between agencies and establishments under the Government and those which are the result of individual effort or under private control.

During the past year the general health service of the Islands has again proved its efficiency and shown progress in many lines of endeavor. No investment pays better dividends to any commonwealth than money expended for the prevention of disease. It is believed that the people of these Islands have come to a full realization of the fact that the health service is their best friend.

The need of physicians in the Islands is emphasized, and endeavor to cause such distribution of available medical men as will bring the greatest good to the greatest number of people is mentioned. A similar condition obtains in regard to the practice of nursing, and the excellent service of our graduate nurses in the Government service and in private practice or employment has led to steps to increase the output of nurses in the Training School for Nurses of the Philippine General Hospital. The es-

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tablishment of a one-year school for midwifery in connection with the Philippine General Hospital is announced.

The extension of hospitals and dispensaries in the provinces is most necessary, and the accomplishment of this purpose is one of the pressing problems now in process of solution.

The continued improvement in general health conditions has received and will receive Governor-General Harrison's most enthusiastic support. Both he and Secretary Denison promise their whole-hearted coöperation in progressive measures for the amelioration of the public health.

PRESIDENT'S ADDRESS: THE MOST IMPORTANT MEDICO-ECONOMIC PROBLEM OF THE PHILIPPINE ISLANDS

By Dr. N. M. Saleeby

Dispensary experience has shown the writer the widespread distribution of subacute, chronic, and recurrent beriberi. In 1907 he examined in one day 64 patients at the dispensary and detected 40 cases with cardiac affections. Within the next few months new and acute cases of beriberi were definitely diagnosed. This latter finding explains the etiology of the cardiac affections noted above. Emphasis is laid on the fact that mild or light symptoms of beriberi, particularly when œdema is not marked, often pass unnoticed by the general practitioner. Earlier observations are corroborated for the coincidence of beriberi and the puerperium after uneventful delivery; the mother may be partially paralyzed. In some cases the paralysis amounted to complete paraplegia, but the majority presented partial paralysis, accompanied by a group of symptoms generally referred to as polyneuritis. The new-born child of such mothers is affected; even subacute attacks, combined with deficiency of milk, suffice to produce the disease in the child. Weaned infants and children of all ages are as apt to contract the disease as adults. Diagnosis of mild cases in children is difficult, and treatment should be encouraged on grounds of suspicion alone.

From data available and from his own experience Doctor Saleeby then reasserts the extensive existence of beriberi in mild form, which though benign in itself considerably lowers the vitality of the individual, reduces his capacity for labor, and puts him at a great disadvantage in combating tuberculosis and other diseases. Further, in certain states of reduced vitality—as in the puerperium and infancy and during famine and hard times—this benign beriberi becomes fatal, spreads rapidly, and does more harm than tuberculosis or any other affection.

After summarizing briefly the literature on the etiology of beriberi and the other deficiency diseases, the writer briefly refers to his success (with Mr. R. R. Williams) in treating human beriberi. He urges the Government control of the milling of rice and the elaboration of the diet of the Filipino through education and by the increased production of potatoes, beans, cattle, goats, and swine.

SCIENTIFIC SESSIONS: NOVEMBER 5, 6, 7

Many of the papers presented before the association will be published in full in the Philippine Journal of Science. Such papers as are not published in full will appear in abstracts.

The following scientific program was presented and carried out in the order indicated:

SECOND SESSION, NOVEMBER 5, 10.30 A. M.

COLLEGE OF MEDICINE AND SURGERY, CALLE HERRAN

Clinicopathological conference.

Drs. B. C. Crowell and W. E. Musgrave.

The technique of the bacteriological diagnosis of cholera (demonstration at the Bureau of Science) ....Dr. Otto Schöbl.

THIRD SESSION, NOVEMBER 5, 4 P. M.

COLLEGE OF MEDICINE AND SURGERY, CALLE HERRAN

Exhibition of specimens from the museum of pathological anatomy, College of Medicine and Surgery, University of the Philippines...Dr. B. C. Crowell.

Bacteriological findings bearing on the epidemiology of cholera.

Dr. Otto Schöbl.

Cholera carriers in relation to cholera control.

Maj. E. L. Munson, U. S. Army.

Cholera at Bilibid..Dr. J. W. Smith.

Observations on cholera in Manila, 1914.

Drs. A. P. Goff and O. S. Denney.

Notes on Philippine water supplies...................Mr. G. W. Heise.

Monstrosities and abnormalities........Dr. Maria Mendoza-Guazon.

FOURTH SESSION, NOVEMBER 6, 7.30 A. M.

PHILIPPINE GENERAL HOSPITAL, CALLE TAFT

Surgical clinic..Dr. P. K. Gilman.

COLLEGE OF MEDICINE AND SURGERY, CALLE HERRAN

Medical and sanitary public welfare work in the Philippine Islands..Dr. L. Booth.

Malaria in the Philippine Islands........................Dr. M. A. Barber.

The sperochætal infection of ulcers in China....Dr. H. E. Eggers.

The treatment of infantile beriberi..........................Dr. José Albert.

The experimental treatment of beriberi with the constituents of rice polishings:

Chemical preparations........................Mr. R. R. Williams.

Case records..Dr. N. M. Saleeby.

The protective properties of normal and of autoclaved milk against beriberi....Drs. R. B. Gibson and Isabelo Concepcion.

Two compounds of emetine which may be of service in the treatment of entamœbiasis..................................Dr. A. G. DuMez.

SIXTH SESSION, NOVEMBER 7, MORNING

Exhibition of a field hospital of the Medical Corps of the United States Army..........................Col. M. W. Ireland, U. S. Army.

[NOTE.—A special car will leave the side track at the Philippine General Hospital at 7.30 a. m. for Fort William McKinley and will return after the exhibition is over so as to arrive at the General Hospital at 10.30 a. m.]

Clinical conference at the Philippine General Hospital.

Drs. W. E. Musgrave, A. G. Sison, and Ariston Bautista.

SEVENTH SESSION, NOVEMBER 7, 2 P. M.

COLLEGE OF MEDICINE AND SURGERY, CALLE HERRAN

Demonstration of electrically heated and regulated apparatus.

Dr. E. H. Ruediger.

Demonstration of pathogenic tropical fungi.

Capt. Ferdinand Schmitter, U. S. Army.

Some pathogenic tropical fungi.

Capt. Ferdinand Schmitter, U. S. Army.

The occurrence of Bacillus coli communis in the peripheral blood of man during life................................Dr. E. H. Ruediger.

The germicidal power of glycerol....................Dr. E. H. Ruediger.

The preparation of antitetanic serum............Dr. E. H. Ruediger.

The bacteriology of leprosy............................Dr. J. A. Johnston.

Schistosomiasis in the Philippine Islands.........Dr. David Roberg.

The development of the eggs of Ascaris lumbricoides.

Mr. L. D. Wharton.

Cæsarean section in the Philippine Islands.

> Dr. Fernando Calderon.

Cæserean section.........................Surgeon Woodward, U. S. Navy.

Cancer of the cœcum with intestinal intussusception.

> Maj. R. M. Thornburgh, U. S. Army.

Liver abscess..............................Drs. P. K. Gilman and J. E. Reed.

BUSINESS MEETING, NOVEMBER 7, 1914

The annual business meeting of the Philippine Islands Medical Association was called to order at 5.30 p. m., President N. M. Saleeby presiding.

The minutes of the previous meeting were read and approved.

The secretary-treasurer reported a balance of 28.07 pesos received from the former acting secretary-treasurer, Elbert Clark, from which certain incidental expenditures had been incurred for announcements made during the year and for the present meeting. He announced that the Manila Medical Society had appropriated 500 pesos for the expenses of the present meeting.

The report of the nomination committee, appointed by President N. M. Saleeby at the opening session, was read and accepted. The following slate was proposed:

For President:	Col. W. D. McCaw, U. S. Army.
For Vice Presidents:	{ Lieut. Col. S. C. Gurney, P. C. { Dr. A. G. Sison.
For Councillor:	Dr. N. M. Saleeby (vice Heiser).

There being no further nominations, the secretary-treasurer was instructed to cast the ballot for the above candidates.

It was moved and seconded that the next annual meeting be held in Manila during the first week of November, 1915, on such days as the council may select. The motion was carried.

It was moved and seconded that the association express its thanks to the proper authorities of the University of the Philippines and of the Philippine General Hospital for the courtesies extended to the association for meeting halls, clinics, etc. The motion was carried.

It was moved and seconded that the association express its thanks to the department surgeon of the Department of the Philippine Islands for the exhibition of the Field Hospital at Fort William McKinley. The motion was carried.

It was moved and seconded that a vote of thanks be given the officers of the association for their work during the year and for the present successful meeting. The motion was carried.

The chairman of the committee on arrangements of the Manila Medical Society moved the following for that committee:

The committee on arrangements for the tenth annual session of the Philippine Islands Medical Association recommends that a vote of sincere appreciation of the association be taken and forwarded to the Manila Electric Railroad and Light Company, through Dr. H. D. Kneedler, for its courtesy to the association in furnishing a special car from the association headquarters to Fort William McKinley and return on the morning of November 7, 1914.

The above motion was seconded and carried.

It was moved and seconded that the following resolution be adopted:

That the thanks of the association are hereby extended to the three American daily papers of Manila for the space they have generously given in their columns to the announcements and program of the association.

This resolution was adopted.

The three following resolutions were read for the committee on public policy and legislation, and these were recommended by the committee for adoption:

Resolved, That in the opinion of the Philippine Islands Medical Association, sufficient evidence has been produced to show that extracts of rice polishings, or tiqui-tiqui, are of undoubted value in the curing of infantile beriberi and of the majority of acute affections of beriberi in adults;

That the association, therefore, strongly recommends the preparation of these extracts on a large scale by the Government of the Philippine Islands and the free distribution in a way to facilitate their acquirement by the public, especially for the poorer classes of the inhabitants of these Islands;

That the commercial manufacture of these extracts be so guarded, controlled, or licensed as to insure their potency, purity, and safety.

Resolved, That the Philippine Islands Medical Association indorses the estimates for appropriation of funds from the Insular Treasury for antituberculous work by the Bureau of Health;

That it further recommends that the aid of the Government be extended to continue the present activities of the Philippine Islands Antituberculosis Society;

That the association believes that every effort should be made for the further extension of antituberculosis work as much as possible.

Resolved, That in the opinion of the Philippine Islands Medical Association sufficient evidence has been produced to show that beriberi occurs frequently in the Philippine Islands in women in the puerperal and nursing life, and through them in their infants, and that this condition is the chief cause of excessive mortality among infants in these Islands;

That, accordingly, special hospital facilities, under the control of the Bureau of Health, should be established to provide the necessary hospital treatment for this class of patients, and for the further study of the disease and its treatment;

That it is further the opinion of this Association that this measure is both serious and necessary, and that it should take precedence of other charities and accessory measures of health.

These resolutions were acted upon individually and were adopted.

On motion, which was seconded, the business session and the annual meeting adjourned at 6.45 p. m.

R. B. GIBSON,
Secretary-treasurer,
Philippine Islands Medical Association.

EDITORIAL

PROGRESS IN THE INVESTIGATION OF VITAMINES

We take this opportunity to pay tribute to the splendid work of Dr. Casimir Funk of the Cancer Hospital Research Institute in London. His discovery of the vitamines of rice polishings, yeast, and other materials during 1911 and 1912 is by far the most notable advance in our knowledge of the deficiency diseases for which any one man has been responsible. The significance of the theory of vitamines is very far-reaching, extending beyond the limits of pathology into the wider and more fundamental fields of nutrition and growth. Whether or not the theory as a whole will stand the test of time, it has already proved its value in stimulating interest and suggesting lines for research.

At the same time we desire to urge upon Doctor Funk the duty of publishing the results of his work more fully and promptly, thus enlisting the energies of workers throughout the world to an early solution of the more practical phases of problems. Workers in the Orient who see thousands of people dying each year from beriberi feel with especial keenness the crying necessity from a humanitarian standpoint of producing vitamines as practical therapeutic agents. Therefore we have been looking eagerly forward to the publication of "the results of their curative power," and "the chemical investigation of all the fractions" [1] (isolated from rice polishings) which were promised in June, 1913, and "a method which will be described later on" [2] (for obtaining the vitamine fraction from yeast) which was referred to a year ago.

R. R. WILLIAMS.

[1] *Journ. Physiol.* (1913), **46**, 179.
[2] Ibid. (1914), **48**, 229.

REVIEW

In recent years the problem of the care of white children in the tropics has become of great importance as the warm countries have been developed and the length of residence in the tropics of men with families has increased.

This book, based upon fifteen years' experience in the treatment of children in the tropics, should be of great assistance to practitioners in a field in which there is comparatively little available literature.

Doctor Harston presents his ideas clearly and convincingly. Four chapters are devoted to the incidence of diseases, climatic factors, considerations of hygiene, and the general welfare of European children; one chapter, to the care of European infants; twenty-one chapters, to pictures of specific diseases as they appear in children in the tropics; and a final chapter, to repatriation.

If, as Sir Patrick Manson says in his introductory remarks, there should be criticism of some of the views expressed in regard to treatment, as is likely to be the case, this may prove to be an important result of the appearance of the book, through leading to the publication of valuable, but hitherto unpublished experiences in the management of the health of white children in the tropics.

MARY POLK.

BOTANY

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EXPERIMENTAL TREATMENT OF HUMAN BERIBERI WITH CONSTITUENTS OF RICE POLISHINGS [1]

By R. R. WILLIAMS and N. M. SALEEBY

(*From the Laboratory of Organic Chemistry, Bureau of Science, Manila, P. I.*)

TWO PLATES

The work which we wish to present was undertaken primarily in the hope that it might lead to some practical suggestions for the treatment of beriberi. In addition, it was felt that the experimental study of the etiology of the disease has been too much limited to polyneuritis gallinarum and that further work on human beriberi would produce more convincing evidence of the nature and causes of the disease. Considerable use has been made of extract of rice polishings in cases of beriberi. It has proved distinctly beneficial in infantile beriberi,[2] but in adults, with few exceptions, the treatment has failed to be of service. Nevertheless the experimental results produced in fowls with certain preparations of rice polishings have been such as to demand a further effort to produce a more widely useful remedy.

Many of the cases that we have treated and observed have been chronic or recurrent, the type of beriberi most obstinate to treat. The number of acute cases in Manila this year (1914), other than among infants, has not been large. As the Liga Nacional para la Proteccion de la Primera Infancia has been carrying on extensive work with infants, we have been forced to accept some cases perhaps not very desirable from

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[1] Chemical preparations by R. R. Williams; case records by N. M. Saleeby. Presented at the annual meeting of the Philippine Islands Medical Association, November 6, 1914.

[2] *Bull. Manila Med. Soc.* (1912), **4**, 26; Albert, *This Journal, Sec. B* (1915), **10**, No. 1.

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the experimental standpoint. However, it may be remarked that the cases we have observed are a fairer average of beriberi as it commonly occurs than an equal number of carefully selected cases.

ALLANTOIN

The extract of rice polishings is a very complex mixture, and the constituents which have shown activity in fowls are present in comparatively minute quantities. Funk's vitamine is generally regarded as the principal active substance. However, this substance is present in a free state in the ordinary extract in vanishingly small amounts, and such an extract utterly fails to cure severely prostrated birds. One of us working with Vedder[3] reported a cure of infantile beriberi with an extract from which the vitamine both free and combined had been completely precipitated and removed with phosphotungstic acid. Allantoin was subsequently found in this extract. It had previously been isolated by Funk[4] by another method, and its curative powers tested on fowls. He reported that allantoin frequently relieved the prostration and prolonged life.[5] This is in general accord with our own results on fowls.

Allantoin is easily prepared synthetically by the oxidation of uric acid with potassium permanganate. Allantoin occurs normally in the urine of many animals. In birds and human beings, according to the best evidence, it is only present to any great extent when it exists as such in the food.[6] From what is known of Funk's vitamine, it seems probable that allantoin resembles this vitamine more or less in its chemical constitution. Allantoin could be placed upon the market at a nominal price, and its purity could be readily and easily controlled. If it should prove beneficial in any considerable proportion of cases, it would be a useful although probably far from ideal remedy. We have treated 5 cases of human beriberi with allantoin, and the records are presented below.

CASE 1

A girl aged 5 years was admitted to the hospital, having been sick for two weeks and was steadily growing worse. The child's sister (case 20) was in much the same condition. The mother, who was nursing an infant 2 months old, was chronic-

[3] *This Journal, Sec. B* (1913), **8**, 192.
[4] *Journ. Physiol.* (1912), **45**, 75.
[5] Ibid. (1912), **45**, 489.
[6] Ackroyd, *Biochem. Journ.* (1911), **5**, 400.

ally beriberic. Œdema in the child was marked and general, with puffiness of the eyelids and marked pitting on the legs. The gait was rather unsteady, the patellar reflexes were absent, and the voice was husky. The face and mucous membranes were anæmic, and the child was very restless and peevish. She suffered from excessive diarrhœa and prolapse of the rectum. The heart showed some enlargement. The first sound was short, and the second was accentuated.

The child was treated for the diarrhœa upon admission, and on the following day administration of 0.1 gram of allantoin, three times daily, was begun. General improvement was noted a day later. In the course of four or five days the œdema had practically disappeared, the gait became steady, the voice clearer, and the color better. The child was also brighter and better contented. The area of heart dullness decreased somewhat, and the sounds improved, although they did not become entirely normal. The patellar reflexes changed very little.

<div align="center">CASE 2</div>

A girl, aged 9 years, was first seen about September 12. She had been sick a month with fever. The gums were spongy and bleeding. She was given a mouth wash, and when again seen on September 22, her gums were improved, but were still dark, swollen, and spongy. The general condition remained much the same as before and may be described as follows:

The face was pale and puffy, especially around the eyelids. The patient appeared fleshy and heavy, but anæmic. Œdema was general, but most marked in the legs. The patellar reflexes were much diminished, the calves were slightly tender, and the gait was wobbly. The lower limbs were numb. The heart showed evidence of palpitation and slight enlargement. The first sound was rough and prolonged, and the second was accentuated. The temperature was 39°. The urine contained albumin and abundant blood cells. A blood examination showed no filaria.

The patient was admitted to the hospital on September 22 and given a cathartic. The following day the fever had disappeared, and treatment with 0.1 gram allantoin four times daily was begun. An improvement in the general condition and gait and diminution of the œdema were noted the following day and continued for a week. The heart condition was also improved considerably after five days, and the patient could walk and run without stumbling, which she had been unable to do at first. The patellar reflexes remained practically un-

changed, and the allantoin was increased to five doses daily. No further improvement was noticeable, and the patient was discharged at the end of twenty-five days. The albumin in the urine had gradually diminished to a trace. The patient seemed practically normal as to the condition of the mouth, the gait, and general comfort, and the œdema had disappeared.

CASE 3

A woman, aged 26 years, was admitted to the hospital with a sick child 2 months old that she was nursing. She dated her illness from about the sixth month of pregnancy and suffered from that time with swollen legs, pains in the calves, and a feeling of weakness. The child was her first. Three days after the birth of the child she felt numbness of the legs and formication, which still continued at the time of her admission to the hospital. She looked weak and anæmic and complained of headache, sleeplessness, sense of heat in the chest, pain in the back, and inability to walk with comfort. The calves of the legs were tender, and the reflexes very slightly sluggish. Œdema of the legs was slight.

The area of heart dullness was very little increased. An indistinct systolic murmur was heard only at the apex, and the second sound was strongly accentuated. Respirations were rapid, deep, and full. A dry musical râle was audible over the apex of the right lung. The patient had suffered from a cough for some months, which appeared to be largely laryngeal. The urine showed no abnormal character.

The patient was given 0.4 gram of allantoin three times daily. She showed no marked improvement in any respect during several days. Later the distress in the chest became more severe, and the heart murmur was more distinct. The allantoin in larger doses seemed to have a depressing effect. It was, therefore, stopped, and digitalin was administered with the usual effects. She was discharged after twenty days without having been materially benefited by the treatment.

CASE 4

A woman, aged 25 years, mother of 2 children, the youngest being a nursing infant of 3 months, was admitted to the hospital. She complained of having felt numbness in the limbs for two months with formication at times. She was pale and anæmic, had little appetite, and felt dizziness and painful oppression in the chest. There was some œdema in the legs, no

œdema in the feet, and the patellar reflexes were exceedingly sluggish. The area of heart dullness was slightly increased; the beat was intermittent and arythmic, the first sound being short and the second rather valvular and accentuated. There was some resonance in the upper lobe of the right lung, but respirations were deep and full.

The patient was given 0.4 gram of allantoin three times daily for three days. There was no apparent improvement other than a more restful appearance. On the fourth day the heart beat became more intermittent and arythmic. Therefore the allantoin was stopped, and strychnine and digitalin were administered. The heart became temporarily regular, and the patient was more comfortable.

<div align="center">CASE 5</div>

A woman, aged 50 years, was admitted to the hospital on August 21 with typical symptoms of mild chronic beriberi, including numbness and heaviness of the legs, tenderness of muscles, sense of heat, and formication, with reflexes diminished in the left knee and exaggerated in the right. Dyspnœa was marked, and the patient tired out very quickly. The second heart sound was valvular and accentuated. The lungs and urine were negative.

The patient was given 0.1 gram of allantoin six times daily for one week without receiving any benefit; on the contrary, if there was any change, the heart condition was slightly worse, and the patient seemed rather better after the allantoin was stopped.

These cases treated and observed by us jointly do not afford definite evidence of any beneficial result of the treatment with allantoin. In those which were distinctly chronic it may be said with certainty that the treatment did not produce any improvement. Rather, there is indication that in large doses allantoin produces a depressing effect upon the heart. In the acute cases there was marked improvement during the period of observation. However, such improvements are not uncommon as a result of a good diet, rest, and care alone.

We are able to present the clinical records of 5 other cases treated with allantoin and observed by other physicians. These cases were treated in their homes without enforcing any change of diet or habits. They indicate that allantoin may frequently relieve the severity of the symptoms in acute cases much as it does in fowls. Further investigation will be necessary to decide

whether or not treatment with allantoin furnishes any substantial advantages as a temporary measure over the symptomatic treatment of acute beriberi commonly in use.

The following two cases were treated and observed by Dr. José Bantug, of Malolos:

CASE 6

Symptoms before the treatment.—The patient was anæmic, with frequent attacks of palpitation and pain in the precardial region; oppression of the chest at times, slight atrophy of the body musculature, formication with slight œdema of the legs; pulse strong and rapid, but regular in rhythm, second pulmonic sound accentuated and the heart slightly enlarged.

After using the allantoin preparation for about a week, the patient was relieved of most of her complaints so much so that further treatment was discontinued.

She is at present attending to her household duties without experiencing the least inconvenience, and all the symptoms have disappeared. She is still slightly thin, but beginning to pick up now.

CASE 7

A man, 21 years old; family and personal history, negative.

Present illness.—About four months ago the patient felt pain and uneasiness in the stomach whenever empty. The pain was relieved on taking food. This pain and distress in the stomach continued daily for the first two and one-half months. One and a half months ago the patient noticed that his abdomen was getting large and his lower extremities were getting swollen. The feet were heavy, and when at home the patient preferred to have them elevated. The lower limbs, sometimes benumbed, are easily fatigued on slight exertion, with profuse clammy sweats. Palpitation is frequent, and without external cause.

June 16.—First examination: Head and neck, negative; chest, coughs a little; lungs, negative; heart, slightly enlarged toward the left; abdomen, large; upper extremities, negative; lower extremities, legs slightly pit on pressure; knee jerks present, but lessened.

Five doses of 0.1 gram allantoin were administered.

June 17.—Stomach feels lighter after meals; pain in the chest; allantoin, 6 doses.

June 22.—No change, except that stomach is better; allantoin, 6 doses.

June 23.—Allantoin, 3 doses. Palpitation less frequent and with less force.

June 29.—Feels well; sweating profuse as formerly.

July 13.—Still with slight palpitation and profuse sweating.

Result of treatment: markedly improved.

Case 8 was treated and observed by Dr. Canuto Reyes.

CASE 8

A woman, aged 33 years, who had given birth to a child nine days previously, was attacked on July 21 with severe pain and numbness in the extremities, and pain and oppression in the chest. When seen the following day, there was marked general œdema, which was especially apparent in the face. The pulse was rapid, and the heart presented palpitations and

an accentuation of the second sound. The knee reflexes were somewhat diminished, and the patient could stand and walk only with considerable pain and difficulty.

Allantoin was administered in doses of 0.1 gram six times daily. The next day toward evening a fever came on, the temperature reaching 40°. All the symptoms were considerably intensified, and the patient was unable to rise from her bed. The allantoin was continued. The fever disappeared during the night, and the following day the patient's condition seemed much better than when first seen. After four days she no longer complained of pains or numbness and was able to walk without difficulty. She continued to nurse her child throughout the treatment.

Ten days later her child fell sick with the usual symptoms of infantile beriberi and was successfully treated with tiqui-tiqui extract. The symptoms, with the exception of œdema, later reappeared in the mother, although much less severely than on the previous occasion.

Case 9 was treated and observed by Doctor Castañeda.

CASE 9

An infant, 2 months of age, had a sudden convulsion on July 16. Slight cyanosis was apparent about the mouth; the pulse was 130; the temperature was normal. The child was uneasy and vomited frequently and was treated with caffein citrate. Four days later the child was paler and more cyanotic, had no appetite, vomited frequently, and the urine was scanty. Attacks of dypsnœa were frequent, and the child cried often. The second heart sound was weak.

The mother was pale and complained of numbness in the legs and pain in the chest. The heart palpitated occasionally; the pulse was 95. There was no œdema nor loss of reflexes. The child was given 0.05 gram of allantoin three times daily, and the mother received 0.1 gram six times daily.

There was a marked improvement in the condition of the child after two days. After eight days the cyanosis and dypsnœa had disappeared, the urine became normal in quantity, and the child's general condition as indicated by the color, activity, and contentment was greatly improved. Improvement in the mother was slight. She continued to nurse the child throughout the treatment.

The record of the following case was furnished by Doctor Elizalde:

CASE 10

Japanese, male, 31 years old, married, a laborer by occupation, residing in Calamba, was admitted to the Philippine General Hospital on July 9, 1914, complaining of numbness in lower limbs and difficulty in walking. Family and past history not reliable, as patient was unable to talk or understand English or Spanish. The present illness began twenty days before admission with vomiting after meals accompanied by numbness and weakness in the lower extremity.

On physical examination the patient was found to be well developed and well nourished; able to walk a few steps with support, but with difficulty. He could not flex the legs nor the feet; heart beats were rapid; cardiac dullness not increased. A soft systolic murmur was heard best in the

mitral area. Knee jerks were absent. There was tenderness in the muscles of the calves when pressed, and the muscles of the hand were very weak.

The day after admission he was given elixir of iron, quinine, and strychnine, 4 cubic centimeters three times a day, without noticeable improvement. This treatment was discontinued July 13, and instead he was given 0.5 gram allantoin powder twice a day.

On July 14 the heart beats were still rapid, but no abnormal murmur was heard, and the patient could walk better than on admission. One centigram of allantoin was given every two hours instead of twice a day.

On July 17 the patient was able to walk without support. Tenderness in the muscles of both calves on pressure still persisted.

HYDROLYZED EXTRACT

Heretofore the only extract of rice polishings used upon human cases has been that prepared with neutral alcohol. Such an extract will produce no prompt effects upon beriberic fowls until it is hydrolyzed. The hydrolysis may reasonably be presumed to break down the complex nitrogenous substances, thus setting free the vitamine together with other cleavage products. Attention has been called before to the alteration so produced in the character of the extract so that it becomes promptly curative, but highly poisonous in excessive doses. The poisonous effect is doubtless due in large part to free choline. We desired to try this extract upon human subjects and adopted the following method with the idea of reducing the amount of free choline as much as possible:

The polishings were extracted with liberal quantities of 20 per cent alcohol, instead of 90 per cent, in order to avoid the solution of much lecithin from which the choline is derived. This extract was concentrated in vacuo and precipitated with strong alcohol. The filtrate was evaporated in vacuo until all the alcohol was removed. The residue was then hydrolyzed by heating five hours on the steam bath with 10 per cent sulphuric acid. The sulphuric acid was removed with calcium carbonate, and the extract was made up to such a volume that 1 cubic centimeter of extract was equivalent to 10 grams of the original polishings. We have found that such an extract can be used safely in proper doses and have treated 8 human cases with it.

CASE 4. FURTHER TREATMENT

Very little permanent improvement having been obtained during several days' treatment with allantoin, the patient was given 15 cubic centimeters of the hydrolyzed extract three times daily. She felt considerably more comfortable and was brighter after two days. On the fourth day of this treatment the patellar

reflexes were found to be more active; the heart sounds themselves were nearly normal in quality, but the arhythmia seemed somewhat increased. This symptom has persisted more or less throughout the treatment with hydrolyzed extract, although the general comfort of the patient has increased, the pain in the legs disappeared, and the sense of heaviness diminished. She nursed her sick child (case 18) throughout these treatments.

<div align="center">CASE 11</div>

A boy of 17 years was admitted on October 16, complaining of numbness, heaviness, and pain in the legs. This condition began six days previously and was becoming worse. He reported having had a slight swelling and numbness of the feet about a year before. The patellar reflexes were diminished, the calves of the legs were tender to pressure, and formication was felt frequently. Œdema was general, but not excessive. He could walk only with much pain and difficulty. The heart was moderately enlarged to the right, the first sound presenting a faint murmur, and the second being accentuated. There was no dyspnœa, and the lungs appeared normal. The urine showed no albumin nor casts.

Treatment was begun with 12 cubic centimeters of hydrolyzed extract three times daily. The dose was increased from time to time to 40 cubic centimeters. The œdema disappeared rather rapidly, and the patient felt much more comfortable. After a week he could walk with comparative ease, could run a little, and stoop and touch the floor with his hands. He stated that the pains in the legs had disappeared, the numbness remaining, however, over a less extended area. The reflexes were more active. After that time improvement was very slow, and when last seen the patient still complained that his knees ached on walking more than a few meters.

<div align="center">CASE 12</div>

A woman, aged 30 years, was admitted to the hospital with a child (case 18) 2 months old that had partially recovered from an attack of infantile beriberi after treatment with tiquitique extract by Doctor Castañeda. The patient dated her illness from the birth of the child, and although she had borne 7 children previously, said she had had no similar illness. She complained of pain and oppression of the chest, numbness and heaviness of the legs, dizziness, and formication. She was pale and anæmic. The knee reflexes were active, and œdema of the legs was slight. The heart was slightly enlarged, the first

sound prolonged and presenting a murmur, the second short, sharp, accentuated, and split. Venous pulsation in the neck was very marked. The urine showed considerable albumin and a few blood casts.

She was given 6 cubic centimeters of hydrolyzed extract three times daily. This was later increased to 10 cubic centimeters. Within a few days the heart condition improved markedly, the splitting of the second sound disappeared, and the murmur became fainter. Otherwise, the patient remained much the same. As there were evident complications, the case was discharged without further treatment.

CASE 13

A woman, aged 16 years, was admitted with an infant 2 months old that had been previously treated with tiqui-tiqui extract and had nearly recovered except for hoarseness of the voice. The child was treated further with tiqui-tiqui extract and gained rapidly in weight and vivacity.

The mother's illness dated from eight days after the birth of the child. She complained of numbness and weakness and pain in the legs, dyspnœa, difficulty in swallowing, and headaches. The patellar reflexes were greatly diminished, and a slight œdema of the legs was apparent. She had had more pronounced swelling previously. The heart was very slightly enlarged; the sounds were forceful and arhythmic, the second being accentuated. The urine showed nothing abnormal.

She was given 15 cubic centimeters of hydrolyzed extract three times daily. Improvement was noticeable in two days and continued for a week. The patient became brighter and was comfortable, and the heart condition became nearly normal. The knee reflexes remained unaffected. The patient continued to nurse her child throughout the treatment.

CASE 14

A woman, aged 22 years, was admitted on October 15 with an infant of 6 weeks (case 20) that had been treated for beriberi and partially recovered. The patient complained of pains in chest and limbs "like rheumatism," and difficulty in walking dating from two weeks before the birth of the child. Formication and numbness were marked and general, but œdema was slight and limited to pitting over the tibia. The knee reflexes were sluggish. The heart was not enlarged, and the sounds were very slightly abnormal. The voice was hoarse. The urine showed no albumin nor casts.

She was given 15 cubic centimeters of hydrolyzed extract three times daily for two weeks. She soon felt and looked much better. The pains disappeared first, and the numbness decreased in area until only the toes remained anæsthetic. The patient could walk much more steadily and comfortably. The heart condition and the patellar reflexes remained unchanged. The patient nursed her infant throughout the treatment.

CASE 15

A woman, aged 30 years, was admitted to the hospital with an infant that showed some evidence of beriberi. The mother complained of numbness of all the extremities and of the body up to the shoulder and of pain in chest and between the shoulder blades. The illness dated from the birth of the child three months previously, although numbness of the legs was experienced after the birth of each of 8 previous children. Of 9 children she had borne, 5 died before reaching an age of 3 months. In each case death was sudden, but the cause was unknown. When first seen the mother felt weak and dizzy. Formication and dyspnœa were marked. The area of heart dullness was increased to right and left. The first sound was short and presented a slight murmur; the second was accentuated and somewhat dull. The knee jerks were diminished. No œdema was apparent. The urine showed no albumin nor casts.

Twenty cubic centimeters of hydrolyzed extract were given three times daily; later the dose was increased to 30 cubic centimeters. The patient felt much more comfortable after treatment for several days, and all the symptoms diminished in severity. She continued to nurse her child during treatment.

CASE 16

An infant of 5 months was admitted with the mother (case 15), who said the child was well. It appeared well nourished, but showed a general œdema over body and face. Its movements were sluggish and rather weak. It was not treated directly, but the mother, who continued to nurse it, was given from 20 to 30 cubic centimeters of hydrolyzed extract three times daily. The child soon became noticeably brighter, stronger, and more active and gained rapidly in weight.

CASE 17

An infant of 2 months was admitted with the mother, who was evidently beriberic. The child had been sick four days, had apparently had colic, and had been cyanotic in the face. The parents had lost their first and only other child by a similar

illness.   They had consulted a physician, who prescribed a sedative without noticeable improvement according to the parents. They said the child had had a convulsion the day before.   The baby was pale, peevish, and weak.   The abdomen was distended, vomiting was rather frequent, and the child was persistently constipated.   There was slight œdema, the face being puffy and the skin pitting slightly on pressure.   The knee jerks were normally active.   The heart beat was rather rapid, but otherwise apparently normal.

The child was given 1 cubic centimeter of hydrolyzed extract three times daily.   The dose was later increased to 3 cubic centimeters.   It continued to nurse at the breast, the mother not being treated at this time.   After three days the child was much improved, ceased to vomit, and became stronger and more active, although there was still more or less colic.   Later it became strong and well and gained 23 ounces in weight during ten days, beginning with the second day of treatment.

All of the cases treated with hydrolyzed extract, except the 2 infants, were chronic or recurrent.   Our experience shows that hydrolyzed extract gives distinct relief in chronic cases and better results than any other line of treatment with which we are acquainted.   We are strongly of the opinion, previously expressed and shared by many others, that such cases can be completely cured, if at all, only by long periods of good care and feeding.   It is quite reasonable to suppose that in these cases permanent and extensive nerve lesions have been produced, the rapid repair of which is out of the question.   The most that should be expected of the hydrolyzed extract to do is to stop the progress of degeneration.

In the infant cases hydrolyzed extract produced apparently complete cures, and may reasonably be expected to be more effective than neutral extract.   The danger of poisoning must, however, always be kept in mind.

UNHYDROLYZED EXTRACT

The following 6 cases were treated with the ordinary neutral extract of rice polishings.   The infantile cases came under our care during the treatment of the mothers and were treated on general principles, not for the value of the experimental results.

CASE 18

An infant of 2 months was admitted with the beriberic mother (case 12), who had partially recovered from an illness previously

diagnosed as beriberi and treated with extract of rice polishings by Doctor Castañeda. When seen by us, the child was somewhat œdematous all over the body, the voice was hoarse and aphonic, and the area of heart dullness was increased slightly. It was very restless and peevish.

After treatment with extract of tiqui-tiqui in quantities from 4 to 24 cubic centimeters daily for a week, the child gradually improved in every respect and seemed quite well, except for a slight huskiness of the voice.

CASE 19

An infant of 2.5 months was admitted on October 5 with its beriberic mother (case 4). The child had been coughing for a few weeks. The throat was a little sore, and the voice was very aphonic. The child was pale. There was general œdema, especially in the legs. The urine was somewhat scanty; the appetite was good, although vomiting after meals was frequent. The heart seemed slightly enlarged downward, but otherwise normal. The patellar reflexes were rather exaggerated.

On October 8 treatment was begun with 1 cubic centimeter of tiqui-tiqui extract four times daily. Improvement was rather slight for about a week, although the vomiting was much diminished. Later a general improvement in the appearance and temper of the child was apparent. The œdema disappeared, the vomiting ceased, and the child became strong, active, and vivacious and gained 11 ounces in weight during the third week. The voice improved very markedly. The child continued to nurse the beriberic mother, who first received allantoin without improvement, and later received hydrolyzed extract with somewhat better results. The later improvement in the child was no doubt in part due to the hydrolyzed extract received by the mother.

CASE 20

An infant of 6 weeks was admitted to the hospital with the mother, who was beriberic (case 14). The infant had had an acute attack of infantile beriberi a week previous to admission. It had been œdematous all over the body, and at times, according to the mother, its limbs had become rigid. Having been treated by Doctor Castañeda with extract of rice polishings before entering the hospital, it was much improved. Only a slight puffiness of the face and a general weakness of movement remained. It was further treated with extract and rapidly gained in strength, weight, and general appearance.

CASE 21

A girl, aged 3 years, was admitted to the hospital on September 22, together with her 5-year-old sister (case 1), who was similarly affected. The mother and grandmother living in the same house were also beriberic. The patient had been sick two weeks and was steadily growing worse. Œdema was general and very pronounced in legs and vulva. The abdomen was distended, and the face was pale and puffy. The patellar reflex was absent in the right knee and diminished in the left. The heart was slightly enlarged, and the sounds were somewhat accentuated. The child also suffered from diarrhœa, and was very peevish.

She was treated for the diarrhœa and was given 3 cubic centimeters of extract of rice polishings four times daily. Very slow but steady improvement was noted from day to day, especially in the brightness and temper of the child and in the diminution of the œdema. The dilatation of the heart decreased and the sounds improved, although the beats became rather rapid and equally spaced. The knee jerks changed noticeably and became normal on the left and slight on the right. Some œdema remained after three weeks of treatment, when the patient was discharged at the request of the parents.

CASE 5 CONTINUED

After having been treated one week with allantoin without benefit, the patient, who still showed the symptoms of chronic beriberi, was given 30 cubic centimeters of unhydrolyzed extract of rice polishings three times daily. This produced marked diarrhœa; so the dose was reduced to 15 cubic centimeters and continued for five days. The patient received no benefit, and the heart condition became markedly worse; we were forced to resort to symptomatic treatment with digitalis and strychnine.

Of the use of neutral extract we have little to remark except the confirmation of the general experience that it is very beneficial in infantile, but not in adult cases, especially those of long standing.

VITAMINE

The vitamine found by Funk in rice polishings was isolated in a moderately pure condition, and this preparation was used in treating 6 cases of human beriberi. The method used for the isolation of the vitamine was that described by Funk,[7] with minor modifications, which experience suggested. The chemical methods will be discussed fully in a later paper.

[7] *Journ. Physiol.* (1911), **43**, 395; (1912), **45**, 75.

CASE 22

An infant of 3 months was admitted on October 2 with its beriberic mother (case 3). It had been sick since September 16, when it had had an apparent convulsion and was treated by a physician. The urine had been suppressed for two or three days following. When first seen, the child was restless, slept badly, and cried incessantly and seemed to have abdominal pains. It vomited frequently after nursing and was constipated. The voice was hoarse and aphonic, the face anæmic and puffy around the eyes. The legs appeared thin, but were slightly œdematous, which condition extended more or less markedly all over the body. It was rather active and vigorous in its movements. The knee jerks were somewhat exaggerated. The area of heart dullness seemed slightly increased. It continued to nurse at the breast, the mother being treated with allantoin.

The next day the infant was given by mouth the vitamine fraction from about 12 kilograms of rice polishings, although it was later discovered that the amount of vitamine present was very small. An equal dose required forty-eight hours to relieve fairly completely the prostration of a neuritic chicken.

Nevertheless, on the following day the child was distinctly brighter, much better contented, stronger and more active. It had 5 bowel movements, but no rise of temperature. It slept quietly, the respirations seemed normal, and the voice was improved. Slight improvement continued for three days, after which treatment was begun with neutral extract. At the end of another week the voice was markedly stronger, the reflexes were normal, and the general condition of the child indicated a complete cure. During the last week the child gained 9 ounces in weight.

CASE 23

An infant of 2 months was admitted on October 22, when the following observations were made. The child seemed weak and had little appetite, had some constipation, and vomited frequently. The mother was beriberic. The child's knee reflexes were practically absent. The extremities were thin, the skin lying in folds. The voice was aphonic, and the heart and lungs were negative.

The mother was given hydrolyzed extract and improved in general condition. The child was not treated, but continued to nurse at the breast. Vomiting ceased after four days, but there was no marked improvement otherwise.

On October 2 at 4 o'clock in the afternoon the child was given

a hypodermic injection of 80 milligrams of crude semicrystallized vitamine, to which some brown sirupy mother liquor still adhered. The temperature rose rapidly within a few hours and reached 40°.3 during the night. After thirty-six hours the temperature had again become normal and has since remained so. Some redness and induration appeared at the point of injection, which has since practically disappeared. Forty-eight hours after the injection the baby was bright and active and seemed quite well. In spite of the period of high fever the child gained 5 ounces in weight in three days after the injection. The gain continued and amounted to 15 ounces at the end of one week.

CASE 24

A baby, 2 months old, was admitted on November 3; it had been sick two weeks. It was pale, cried frequently, and appeared to have pain in the stomach; it had vomited often for several days. There was slight dyspnœa, and the movements of the child were slow and weak. The heart beats were rather forceful and equally spaced. The mother was beriberic.

At 4 o'clock in the afternoon of November 3 the child was given the semicrystallized vitamine obtained from 10 kilograms of rice polishings. It vomited copiously immediately, and a little later the dose was repeated. The temperature rose after a few hours, reaching nearly 39°, and then subsided gradually. The next day the child's condition had changed radically. It had not vomited since taking the vitamine and appeared well, bright, and contented. It gained 29 ounces in weight during a week, although it had appeared well nourished from the first.

CASE 25

A well-built athletic man, aged 20 years, was admitted to the hospital after a month's illness, previous to which his personal history appeared negative. His illness began with heaviness and numbness of the legs. Later, formication, tenderness of the muscles, general œdema, paræsthesia, and weakness appeared. When first seen, the heart showed a readily visible diffuse impulse in the fourth and fifth interspaces. The area of heart dullness was increased upward and downward, a little to the left but most markedly to the right. A faint diffuse systolic murmur and accentuation of the second sound were audible. There was marked visible pulsation in the veins of the neck, and the pulse was of the water-hammer type and easily compressible. The lungs were negative. The calves were markedly tender, and the

knee reflexes were sluggish. The patient walked with difficulty on account of pain in the calves. Œdema was scarcely notice-able. The patient was very querulous and depressed.

He was given in the abdomen a subcutaneous injection of the semicrystallized vitamine obtained from 20 kilograms of rice polishings. Within a few minutes he complained of being unable to see well, and later of dullness of all the senses and severe pain in the abdomen. As the patient was in a very timid and excit-able condition, perhaps no great importance should be attached to his statement of symptoms. There was a very slight rise of temperature.

Little change was observable in the patient's condition until the second day after treatment. On the third day the change was very marked. The visible heart impulse had almost disap-peared, and the dilatation had greatly diminished. The murmur and accentuation of the second sound were no longer heard. The pain in the muscles was very slight, and the patient could walk with ease and could stoop, flexing the knees without pain. The knee reflexes also became slightly more active, although not quite normal. Especially noticeable was the change in the patient's spirits. He continued to improve for a week, and when last seen, a month after treatment, he said that he felt quite well and strong.

<div align="center">CASE 26</div>

An infant, 40 days old, was examined and treated at home with the coöperation of Dr. José Albert. The general appearance of the child indicated that it might have been premature. It was small, weighing 2,500 grams. The arms and legs were thin, the skin lying in folds. The mother showed no very marked symp-toms of beriberi, although the knee reflexes were sluggish. She had had 9 children previously, 7 of whom had died at less than 3 months of age. The eighth was living and well. The child had been sick two days. Dyspnœa was very severe at times, the respirations reaching 140 and the heart beats 200 or more per minute. The pulse was scarcely detectable. Vomiting had not been frequent, and œdema was slight. The voice was husky, the face cyanotic, and the child was very restless and fretful. The symptoms presented were such that death was to be expected hourly.

The child was given by mouth the semicrystallized vitamine from 10 kilograms of rice polishings in 2 doses at 6 and 11 p. m. About one fourth of this was lost in administration. There was no rise of temperature, and improvement in the condition of the

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patient was noticeable before the administration of the second dose, the dyspnœa having become less severe. The child had also eaten with improved appetite. The following day the child breathed easily and freely, and the heart was vastly improved. The appetite was good, and sleep was sound and unbroken. All the symptoms of infantile beriberi disappeared in three days. The gain in weight during a week after treatment amounted to 7 ounces. A month later the child was well and had gained greatly in general robustness.

<div align="center">CASE 27</div>

A boy, aged 9 years, was admitted to the Philippine General Hospital under the care of Dr. José Albert, who kindly invited us to treat the patient and observed the results with us. Earlier than one week previous to admission the family and personal history appeared negative. At this time œdema began to appear in the face and three days later in all extremities. About the same time there was numbness and heaviness of the legs and difficulty in walking. Marked dyspnœa and pains in the chest began the day previous to admission. All of these symptoms were increased in severity, so that when the patient was first seen he was unable to stand or walk. The heart showed general and marked enlargement. The first sound lacked clearness, but was without definite murmur, and the pulmonic second was accentuated. The apex beat was diffused, and pulsation of the neck veins was visible. The muscles of the calves were sensitive to pressure, and the knee jerks were entirely absent. The urine proved negative for albumin and casts.

He was observed for thirty-six hours, being treated with nitroglycerin several times. On the second day after admission, December 8, there was no substantial change in the condition of the patient. Œdema was more marked, and the pulse and respirations were very slightly improved. The heart was still much enlarged, as determined by percussion and verified by a skiagram (Plate I).

He was given at this time the semicrystallized vitamine obtained from 25 kilograms of rice polishings. This was given by mouth during the course of the following twelve hours, being divided into 4 equal doses. A scarcely appreciable rise of temperature occurred. Twenty-four hours after administration of the first dose marked improvement of the dyspnœa and heart condition was apparent. The œdema was also much diminished. At the end of forty-eight hours after the first dose the œdema

had practically disappeared, the heart enlargement had markedly diminished, especially on the right (Plate II), and the general condition was vastly improved, as indicated by the temper, appetite, and voice. Improvement continued for a few days, when the child's condition appeared normal, except for the continued slight accentuation of the pulmonic second and absence of knee jerks. At the end of a month the heart had become entirely normal, and the knee reflexes were detectable, although still much diminished.

In 5 of the 6 cases treated with vitamine, improvement was so prompt and radical as to leave no doubt of the specific curative properties of the substance. Even in the sixth (case 25), although the dose was relatively minute, amounting to about 0.25 gram of dry substance for a man weighing about 50 kilograms, improvement although gradual was marked.

We are unable to assign a definite cause for the rise of temperature after the administration of vitamine. No such temperature rise has been noted in fowls, nor is there any record of an observation to the contrary. Therefore this reaction may be due to the vitamine itself or to an impurity in our product. Funk[8] has noted that the pure substance is without marked physiological properties. If, therefore, the temperature reaction is due to the vitamine itself, it must constitute a specific reaction for beriberi. It will be noted that in the cases in which the temperature rise was slight or absent the amount of vitamine was either small or administered in small doses at intervals of a few hours. This was done to avoid the rise of temperature, which had caused considerable anxiety, especially in case 23. It should be noted also that case 4, after treatment for three weeks with hydrolyzed extract, also experienced a temporary rise of temperature very much like that noted in case 23.

CONCLUSIONS

Allantoin has a beneficial effect in certain cases of beriberi, although probably never amounting to a complete cure. Its value should be tested further.

Hydrolyzed extract of rice polishings has benefited all the types of beriberi upon which it has been tried. It can be of practical service, but should be used only in cases under the direct supervision of physicians and nurses.

Unhydrolyzed extract of rice polishings is a safe and valuable remedy for infantile beriberi, but is of little use for older cases.

[8] *Journ. Phys.* (1913), 46, 173.

The vitamine of rice polishings possesses specific and prompt curative properties far beyond those of any other known substance. Unfortunately its cost at present prohibits its general use among the poorer classes, who are the chief sufferers from beriberi.

As a whole, our observations on the 27 cases recorded in this paper in their bearing on the etiology of beriberi are in accord with the broad proposition that the disease, in a practical sense at least, results primarily from a poor diet, deficient more particularly in specific substances of the nature of Funk's vitamine. The fact that so-called beriberi cases of whatever type respond in a greater or less degree to the same treatment would indicate that they are in reality one and the same disease. We believe that practically all the neuritis which is very prevalent among Filipinos, except, of course, a comparatively small percentage of cases for which some other well-known cause is assignable, may safely be regarded as beriberi.

In conclusion, we are happy to have this opportunity to thank the physicians whose names have been mentioned above, as well as officials of the Bureau of Health and the Liga Nacional for their very kind coöperation in securing and observing cases.

ILLUSTRATIONS

[Skiagrams by Fernandez.]

PLATE I. Case 27. Showing the heart immediately before the administration of vitamine.

PLATE II. Case 27. Showing the heart forty-eight hours after Plate I was taken.

PLATE I.   CASE 27. THE HEART IMMEDIATELY BEFORE THE ADMINISTRATION OF VITAMINE.

PLATE II.   CASE 27. THE HEART FORTY-EIGHT HOURS AFTER PLATE I WAS TAKEN.

THE THYMUS GLAND IN BERIBERI [1]

By R. R. WILLIAMS and B. C. CROWELL

(From the Laboratory of Organic Chemistry and the Biological Laboratory, Bureau of Science, Manila, P. I.)

Funk and Douglas [2] have shown that, among the changes which take place in pigeons suffering from polyneuritis as a result of an exclusive white-rice diet, a marked diminution in size occurs in the glands of internal secretion. Microscopically there is a marked degenerative change of the cells with higher functions. In most cases the marked atrophy is due to a disappearance of the cells, the framework of the gland alone remaining. The most marked change is in the disappearance of the thymus; microscopically no thymus could be seen in any of the beriberic pigeons examined.

Following these observations, a theory that a severe change in the glands of internal secretion occurs in beriberi has been proposed by Funk on the a priori grounds that the vitamines of the food have a close relationship to the glands of internal secretion.

Funk and Douglas omitted to mention the age of the pigeons used in their work, and it seems that this is a factor of supreme importance. In human beings the thymus gland normally undergoes involution after puberty and is also subject to so-called "accidental" involution in the course of both acute and chronic diseases. It is known that normal involution of the thymus occurs in the chicken, but we know of no evidence to prove that "accidental" involution occurs. To assume the occurrence of accidental involution in any individual case without a knowledge of the age of the animal seems erroneous.

In man, in so far as infantile beriberi is concerned, it is known that accidental involution of the thymus does not always occur, and one of us [3] has drawn attention to the occurrence of enlarged thymus in some cases of infantile beriberi associated with status thymico-lymphaticus.

In adult beriberic cases at autopsy our records show that the

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[1] Received for publication November 16, 1914.
[2] *Journ. Physiol.* (1914), 47, 475.
[3] Crowell, B. C., *This Journal, Sec. B* (1913), 8, 77.

thymus gland is usually "small," but one case was found with a thymus gland weighing 28 grams in a child 13 years old (2584), and one weighing 12 grams in a case 18 years of age (2645).

In view of the extreme importance of the subject and the unknown factors involved, it was deemed advisable to record some observations made by us in the course of other work, as to the atrophy of the thymus in cases of beriberi.

In addition, it having been reported that the administration of thymus nucleic acid produced marked improvement in birds suffering from polyneuritis,[4] a further study of thymus tissue seems advisable. Extracts of thymus tissue give the blue color reaction with phosphotungstic acid and alkali[5] to a marked degree, making the existence of vitamines in the tissue most probable. It seemed possible that the thymus might constitute a store of vitamines in the body to an extent out of proportion to the size of the organ. Some color was lent to this view by the fact that young fowls, in which the thymus is normally large, are slightly more resistant to the onset of acute symptoms of polyneuritis than full-grown ones, and that only a much modified form of beriberi occurs in human infants. On the other hand, any effect the thymus tissue may have upon the onset of polyneuritis may be due to purine and pyrimidine bodies. The results which we have obtained with thymus tissue are preliminary, and more definite decision awaits the isolation of vitamines from the tissue.

AUTOPSY FINDINGS

Three supposedly normal pigeons were procured alive in the market and were killed at once. Their age was unknown. The thymus gland of one was relatively very small; another, small; and another, large. In 4 beriberic pigeons which had been fed on white rice the thymus had completely disappeared in every case, as reported by Funk and Douglas.

However, upon examining 16 chickens in which polyneuritis had developed as a result of a white rice diet, it was found that the thymus had completely disappeared in 7 cases, was considerably atrophied in 5 other cases, and apparently was slightly, if at all, altered in the remaining 4. The disappearance of the thymus, therefore, is not a necessary concomitant of polyneuritis in chickens, although it may occur frequently.

[4] Funk, *Journ. Phys.* (1912), 45, 491.
[5] Folin et al., *Journ. Biol. Chem.* (1912), 11, 265; (1912), 13, 363.

That this atrophy is not due simply to the age of the birds is shown by the fact that it took place in half-grown as well as in full-grown fowls.

No relationship could be established between the atrophy of the thymus and the length of the incubation period or the duration, severity, or specific symptoms of the disease. However, our study of the symptoms was not sufficiently minute to exclude the possibility of the existence of such relationship.

The thyroid was also examined in the 16 fowls mentioned, and results similar to those of Funk and Douglas were noted.

Four chickens which had been fed on milk and white rice for varying periods in the course of another experiment were examined after death. These chickens developed evidence of neuritis and were killed. Their sciatic nerves showed microscopic evidences of degeneration in Marchi preparations. The thymus of 1 fed with autoclaved milk and white rice was small. The thymus glands of the other 3 chickens which were fed on whole fresh milk and white rice were large.

The results of all of these examinations are given in Table I.

ADMINISTRATION OF THYMUS TISSUE

Two fowls were fed on white rice with an addition of 10 milligrams of dried sheep's thymus daily. It was estimated that the quantity of thymus tissue ingested during the normal period of incubation would be the same as that normally present in young fowls. This small amount of tissue noticeably retarded, but did not prevent, the onset of the disease.

Two fowls were fed on white rice with a daily dose of the alcoholic extract of 1.5 gram thymus gland. Here again the protection was not complete, although the loss in weight and the onset of the disease were retarded.

Two fowls were fed in the same manner, but with a daily dose of the extract of 3 grams of thymus with less protective results.

For comparison 2 fowls were fed on white rice and 2 milligrams of uracil daily. One contracted chicken cholera as shown by a blood smear. The other was apparently partially protected by the uracil.

Five fowls suffering from polyneuritis were treated with hydrolyzed extract of thymus gland in doses of from 5 to 50 grams of the gland. No cures were obtained.

Two human cases of beriberi were treated with small quantities of thymus, and a slight improvement was shown in each case. This improvement did not continue after the first few

TABLE I.—*Autopsy findings in pigeons and chickens fed on milk and white rice.*

Animal.	Neuritis.			Cause of death.	Diet and treatment.	Thymus, with microscopic control.
	Developed within—	Duration.	Severity.			
	Days.	*Days.*				
Normal pigeon				Killed		Very small.
Do				...do		Large.
Do				...do		Small and of involuting type.
Chicken	23	3	+	Polyneuritis	White rice only	None.
Do	27	2	+++	Choline poisoning	...do	Do.
Do	29	3	++	Polyneuritis	...do	Do.
Do	33	36	+	...do	...do	Do.
Do	(?)	10	+++	...do	...do	Normal (?) amount.
Do		40	+	...do	...do	Small.
Do	15	21	+++	...do	...do	Fairly large.
Do	17	3	+++	...do	...do	Very small.
Do		1		...do	...do	None.
Young chicken	13	3	+	...do	...do	Very small.
Do	16	5	+	...do	...do	None.
Chicken		30	++	Muscarine poisoning	White rice plus 2 doses nicotinic acid	Moderate.
Do		21	++	...do	...do	Fairly large.
Do	27	9	+++	Polyneuritis	White rice plus 2 mg. nicotinic acid daily	None.
Do	30	8	++	...do	...do	Do.
Do	31	3	+++	...do	White rice plus 10 mg. thymus daily	Small.
Do	31	5		...do	...do	Moderate.
Do	16	12		...do	White rice plus 3 doses extract of thymus	None.
Do	26	9		...do	White rice plus 5 mg. allantoin daily	Do.
Do	37	5		...do	White rice plus 5 mg. uracil daily	Do.
Do	30	3	++	Killed	Autoclaved milk plus white rice	Small.
Do	30	5	+	...do	100 cc. fresh milk plus 40 gms. white rice	Large.
Do	64	1	+	...do	...do	Do.
Do	90	1	++	...do	...do	Do.

days of treatment, and the patients at the close of the treatment still showed the characteristic symptoms of the disease, although in a less distressing form.   Inasmuch as it was not practicable to place these cases in a hospital for accurate observation during treatment, the results are of more or less doubtful value, but are given here for what they may be worth.   The dose used was 0.3 gram of dried sheep's thymus six times daily.   No change was made in the diet of the patients.

CONCLUSIONS

The experimental evidence indicates that (1) there is no apparent fundamental connection between beriberi and the atrophy of the thymus; (2) when the latter occurs in birds fed on polished rice, as it frequently does, it is due to some other cause; (3) the thymus gland contains no extraordinary amount of vitamine, and the protective effect of administering the tissue is probably largely due to purine and pyrimidine derivatives; (4) the presence of a comparatively large amount of thymus gland in young animals does not appear to be responsible for their modified susceptibility to beriberi.

We feel that the experimental evidence presented by Funk and Douglas is far too meager to warrant any positive conclusions, much less forming any useful theory regarding the rôle of the vitamines in the body.

TABLE II.—*Thymus-feeding experiments.*

Fowl No.	Addition to daily diet of 50 grams of white rice.	Time required to develop neuritis.	Length of life.	Loss in weight.	
				Total.	Daily.
		Days.	*Days.*	*Per ct.*	*Per ct.*
5	+ 10 mg. thymus	34	37	28.5	0.77
6	do	32	37	33.7	0.91
20	+ 1.5 gm. thymus gland	45	46	20.8	0.57
21	do	52	58	20	0.66
36	3 gm. thymus gland	25	29	31.9	1.57
38	do	31	35	27.5	1.25
8	2 mg. uracil	38	43	36.6	0.85
Average of 6 controls		17.5	21	26.9	1.41

PRACTICAL EXPERIENCE WITH SOME ENRICHING MEDIA RECOMMENDED FOR BACTERIOLOGICAL DIAGNOSIS OF ASIATIC CHOLERA [1]

By Otto Schöbl

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

It appears from the literature as though the classical Koch-Durham peptone solution has not always given satisfactory results. Ohno (1), in this laboratory, made a thorough study of the relation between the chemical reaction of the culture medium and the morphology of cholera vibrio. Led by the experience that a sudden change of chemical reaction of the medium in which cholera vibrio is growing causes change in morphology, he tested the reaction of a series of cholera fæces. As a consequence of this study Ohno advises the use of peptone of three different reactions which correspond to the reaction of the cholera fæces: namely, 0, 3, -0, 5, -1, 3. The significance of this phenomenon in relation to our method of examinations is evident, as the presence of vibrios in the peptone culture was used as an indicator in the search for contact carriers, and only those samples were plated which contained vibrios. The objection may be made that owing to the difference between the reaction of the fæces and that of the peptone medium the cholera vibrios assumed an atypical form and remained unrecognized. It is an actual fact that cholera vibrios will lose the typical vibrio shape if transferred from acid to alkaline medium or vice versa, but such a change is not a permanent one, and the new generations, which follow in rapid succession, adapt themselves to the new conditions and soon appear in typical vibrio shape. This is particularly true of liquid media, provided the reaction remains within the limits of maximal acidity and maximal alkalinity.

Numerous stools from patients, convalescents, and suspects submitted by the quarantine hospital, which were all examined by hanging drop, enrichment process, and Dieudonné's plates, showed that in every instance in which cholera vibrios were found on the plates motile vibrios were present in the corresponding peptone culture after an incubation of from twelve to eighteen hours. All liquid stools from cholera patients were of pronounced alkaline reaction to litmus paper.

Several suggestions recently have been made to substitute for

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the peptone solution a medium which would act not only as an enriching medium, but also as a selective one. The bile medium of Ottolenghi and the application of the principle of Dieudonné's agar to liquid media as suggested by Kraus are particularly of note. The literature on this subject is not lacking in criticism in regard to those two media. Contradictory results have been obtained by various authors,[2] and the impression seems to prevail that the advantages attached to either one of these enriching media are so slight as not to warrant a substitution for the peptone solution. From the purely scientific standpoint the tendency is to cultivate bacteria on media of simple and uniform composition, while from the practical standpoint a reliable medium is desired which can be prepared with the least loss of time and with the least trouble. None of the substitutes exceeds the peptone solution in either of the requirements; besides, as will be seen later, their reliability under certain conditions is doubtful. Owing to the lack of uniformity in their composition, the media must be tested before use.

CULTURAL EXPERIMENTS WITH THE CHOLERA VIBRIO AND BACTERIA ASSOCIATED IN CHOLERA STOOLS

In a series of experiments I have used various enrichment media to compare their practical value—peptone solution of varying chemical reaction (NaOH, Na_2CO_3), Kraus's medium, and the bile medium. Several instances of these comparative tests are given in the tables.

The arrangement of the experiments is evident from the protocols. Liquid stools were used. The stool specimens were thoroughly shaken before planting. The platinum loop was of uniform size (4 millimeters in diameter).

Terms used in the protocols.

—	No cholera colonies present.
=	No growth at all.
+	Cholera colonies present.
Very few.	Less than half a dozen.
Few.	About one dozen.
More than a few.	About 50.
Numerous.	About 200.
Very numerous.	More than 200.

PROTOCOLS OF EXPERIMENTS

One loopful of cholera fæces was inoculated into each tube of enrichment medium. Streak cultures were made on agar plates. One loopful was transferred.

[2] See references at the end of this paper.

TABLE I.—*Enrichment cultures incubated eighteen hours.*

Enrichment.		Growth on plates. Lactose agar.	Remarks.
Medium.	Reaction.		
Peptone............	+2	—	
Do	+1.5	—	Mostly acid producers present besides cholera.
Do	+1	--	
Do	+0.5	+	
Do	—0.5	+	Their number decreases toward the alkaline end of
Do	—1	+	the series of peptone. Plates made from peptone
Do	—1.5	+	+2, +1.5, and +1 overgrown by acid producers.
Do	—2	+	

TABLE II.—*Same arrangement of experiment as in Table I.*

Enrichment.		Growth on plates. Dieu- donné's agar.	Remarks.
Medium.	Reaction.		
Peptone............	+2	+	
Do	+1.5	—	
Do	+1	—	Overgrown by a motile bacillus growing fairly well
Do	+0.5	—	on Dieudonné's plates. One single colony found
Do	—0.5	—	on the plate made from +2 peptone. It proved
Do	—1	—	to be one of cholera vibrio.
Do	—1.5	—	
Do	—2	—	

TABLE III.—*Same arrangement as Table II. Both lactose agar and Dieu-donné's plates used. Incubation of peptone culture, eighteen hours.*

Enrichment.		Growth on plates.		Remarks.
Medium.	Reaction.	Lactose agar.	Dieudon-né's agar.	
Peptone............	+2	—	....	Few acid producers from +2. The num-
Do	+1.5	+	....	ber of red colonies decreases toward the
Do	+1	+	....	alkaline end of series. No blue colonies
Do	+0.5	+	....	except cholera.
Do	—1	+	....	Cholera colonies most numerous on plates
Do	—1.5	+	....	made from peptone of from —0.5 to —1.5
Do	—2	+	....	reaction.
Do	+2	....	+	Only one cholera colony +2 plate. Plates
Do	+1.5	....	+	show pure growth of cholera.
Do	┤1	....	+	
Do	+0.5	....	+	
Do	—0.5	....	+	Cholera colonies most numerous on plates
Do	—1	....	+	made from peptone of from —0.5 to — 1.5
Do	—1.5	....	+	reaction.
Do	—2	....	+	

TABLE IV.—*Same arrangement as Table III.*

| Enrichment. | | Growth on plates. | | Remarks. |
Medium.	Reaction.	Lactose agar.	Dieudon-né's agar.	
Peptone	+2	—		Reaction +2 exclusively red colonies. Red colonies decrease toward the alkaline end of the series but are still present on the plate made from peptone —1.5.
Do	+1.5	+		
Do	+1	+		
Do	+0.5	+		
Do	—1	+		
Do	—1.5	±		
Do	—2	+		
Do	+2		=	From +2 to —0.5 cholera pure; from —1 to —2 besides cholera colonies also small yellowish colonies present. Their number increases with increasing alkalinity of peptone.
Do	+1.5		÷	
Do	+1		+	
Do	+0.5		+	
Do	—1		+	
Do	—1.5		÷	
Do	—2		+	

TABLE V.—*Same arrangement as Table IV.*

| Enrichment. | | Growth on plates. | | Remarks. |
Medium.	Reaction.	Lactose agar.	Dieudon-né's agar.	
Peptone	+2	—		Exclusively red colonies in +2. More red colonies than cholera in —0.5. On —2 plate mostly cholera colonies; only very few red colonies. No blue colonies except cholera.
Do	+1.5	0		
Do	+1	0		
Do	+0.5	+		
Do	—0.5	+		
Do	—1	0		
Do	—1.5	0		
Do	—2	+		
Do	+2		=	Pure cholera. Cholera colonies most numerous from —0.5 to 1.5 reaction.
Do	+1.5		0	
Do	+1		0	
Do	+0.5		÷	
Do	—0.5		+	
Do	—1		0	
Do	—1.5		0	
Do	—2		+	

TABLE VI.—*Same arrangement as Table V. Plates inoculated after six hours' and eighteen hours' incubation.*

SIX HOURS' INCUBATION.

Enrichment.		Growth on plates.		Remarks.
Medium.	Reaction.	Lactose agar.	Dieudon-né's agar.	
Peptone	+2	—		Few red colonies; no cholera.
Do	+1.5	—		Overgrown with red colonies; no cholera.
Do	+1	—		Do.
Do	+0.5	—		Do.
Do	−0.5	+		More than a few cholera colonies. Red colonies predominate.
Do	−1	+		Same as previous, only cholera colonies more numerous.
Do	−1.5	+		Very numerous cholera colonies; few small white and yellow colonies.
Do	−2	+		Numerous cholera colonies; few white and yellow colonies.

EIGHTEEN HOURS' INCUBATION.

Peptone	+1	—		Exclusively red colonies.
Do	+0.5	—		Do.
Do	−0.5	+		Few cholera colonies; red colonies predominate.
Do	−1	+		Numerous cholera colonies; only few red ones.
Do	−2	+		Do.
Do	+1		=	
Do	+0.5		+	Very few cholera colonies, pure.
Do	−0.5		+	Numerous cholera colonies, pure.
Do	−1		+	Very numerous cholera colonies, pure.
Do	−2		+	Same as − 1.

TABLE VII.—*Same arrangement as Table VI. Plates inoculated after six hours' and eighteen hours' incubation.*

SIX HOURS' INCUBATION.

Enrichment.		Growth on plates.		Remarks.
Medium.	Reaction.	Lactose agar.	Dieudon-né's agar.	
Peptone	+2	—		One *subtilis*-like colony; no cholera.
Do	+1	—		Mostly red colonies; few blue spreading colonies; no cholera.
Do	+0.5	+		Overgrown by red colonies; more than a few blue spreading colonies; few cholera.
Do	−0.5	+		Only few red colonies; numerous blue spreading colonies more than a few cholera.
Do	−1	+		Overgrown by blue spreading colonies; more than few cholera colonies.
Do	−2	+		Same as − 1.

TABLE VII.—*Same arrangement as Table VI. Plates inoculated after six hours' and eighteen hours' incubation*—Continued.

EIGHTEEN HOURS' INCUBATION.

Enrichment.		Growth on plates.		Remarks.
Medium.	Reaction.	Lactose agar.	Dieudon-né's agar.	
Peptone	+1	—	----------	Mostly red colonies; few blue spreading ones; no cholera.
Do	+0.5	—	----------	Same as + 1.
Do	—0.5	+	----------	Numerous cholera; few red colonies; few blue spreading colonies.
Do	—1	+	----------	Very numerous cholera colonies; 12 red ones; more than a few blue spreading colonies.
Do	—2	+	----------	No red colonies; more than a few blue spreading colonies; more than a few cholera.
Do	+1	----------	=	No growth.
Do	+0.5	----------	+	Seven cholera colonies, pure.
Do	—0.5	----------	+	Numerous cholera colonies, pure.
Do	—1	----------	+	Very numerous cholera colonies, pure.
Do	—2	----------	+	More than few cholera colonies, pure.

TABLE VIII.—*Same arrangement as Table VII. Kraus's and Ottolenghi's media included. Plates inoculated after six hours' and eighteen hours' incubation.*

SIX HOURS' INCUBATION.

Enrichment.			Growth on plate.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	+1	----------	=	--------	No growth.
Do	+0.5	----------	—	--------	Red colonies exclusively.
Do	—0.5	----------	+	--------	Few red colonies; numerous cholera colonies.
Do	—1	----------	+	--------	Very numerous cholera colonies; about a dozen white ones.
Do	—2	----------	+	--------	Numerous cholera colonies; about a half-dozen white ones.
Kraus's	----------	5	+	--------	More than a few cholera colonies; same number of red ones.
Do	----------	10	+	--------	More than a few cholera colonies, pure.
Do	----------	15	+	--------	No growth.
Do	----------	20	=	--------	Do.
Ottolenghi's	----------	----------	=	--------	Do.

TABLE VIII.—*Same arrangement as Table VII. Kraus's and Ottolenghi's media included. Plates inoculated after six hours' and eighteen hours' incubation*—Continued.

EIGHTEEN HOURS' INCUBATION.

Enrichment.			Growth on plate.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	+1		—		Very few red colonies; no cholera.
Do	+0.5		—		More than a few red colonies; no cholera.
Do	—0.5		+		Numerous cholera colonies; about a dozen red ones.
Do	—1		+		Same as —0.5.
Do	—2		+		More than a few cholera colonies; very few red ones.
Kraus's		20	—		Two blue colonies; no cholera.
Do		15	—		Small white colonies; no cholera.
Do		10	+		More than a few cholera colonies, pure.
Do		5	+		Numerous cholera colonies; very few red ones.
Ottolenghi's			+		Few cholera colonies, pure.
Peptone	+1			=	
Do	+0.5			+	About a dozen cholera colonies, pure.
Do	—0.5			+	Numerous cholera colonies, pure.
Do	—1			+	Same as —0.5.
Do	—2			+	More than a few white cholera colonies, pure.
Do		20		=	No growth.
Do		15		=	Do.
Do		10		+	More than a few cholera colonies, pure.
Do		5		+	Numerous cholera colonies, pure.
Ottolenghi's				+	Few cholera colonies, pure.

TABLE IX.—*Same arrangement as Table VIII. Plates inoculated after six hours' and eighteen hours' incubation.*

SIX HOURS' INCUBATION.

Enrichment.			Growth on plate.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	+1		—		Two red colonies; no cholera.
Do	+0.5		—		More than a few red colonies; no cholera.
Do	—0.5		+		Numerous cholera colonies, pure.
Do	—1		+		Very numerous cholera colonies, pure.
Do	—2		+		More than a few cholera colonies, pure.
Kraus's		20	=		No growth.
Do		15	=		Do.
Do		10	+		More than a few cholera colonies, pure.
Do		5	+		Numerous cholera colonies, pure.
Ottolenghi's			=		No growth.

TABLE IX.—*Same arrangement as Table VIII. Plates inoculated after six hours' and eighteen hours' incubation*—Continued.

EIGHTEEN HOURS' INCUBATION.

Enrichment.			Growth on plate.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	+1		—		Red colonies exclusively; no cholera.
Do	+0.5		+		More than a few cholera colonies, pure.
Do	—0.5		+		Same as +0.5.
Do	—1		+		Numerous cholera colonies, pure.
Do	—2		+		More than a few cholera colonies; few small white colonies.
Kraus's		20	=		No growth.
Do		15	—		Few small white and yellow colonies; no cholera.
Do		10	+		Few cholera colonies; same number of small white colonies.
Do		5	+		Same as 10 per cent.
Ottolenghi's			—		Few yellow colonies; no cholera.
Peptone	+1			=	No growth.
Do	+0.5			=	Do.
Do	—0.5			+	Numerous cholera colonies, pure.
Do	—1			+	Very numerous cholera colonies, pure.
Do	—2			+	Numerous cholera colonies, pure.
Kraus's		20		=	No growth.
Do		15		=	Do.
Do		10		+	More than a few cholera colonies, pure.
Do		5		+	Same as 10 per cent.
Ottolenghi's				=	No growth.

TABLE X.—*Same arrangement as Table IX. Plates inoculated six hours and eighteen hours after incubation.*

SIX HOURS' INCUBATION.

Enrichment.			Growth on plates. Lactose agar.	Remarks.
Medium.	Reaction.	Concentration.		
		Per cent.		
Peptone	+1		=	No growth.
Do	+0.5		—	Numerous red colonies; no cholera.
Do	—0.5		+	Numerous cholera colonies; more than a few red ones.
Do	—1		+	Very numerous cholera colonies; more than a few red colonies.
Do	—2		+	Numerous cholera colonies; few red colonies.
Kraus's		20	=	No growth.
Do		15	–	Do.
Do		10	!	More than a few cholera colonies, pure.
Do		5		Numerous cholera colonies; same number of red ones.
Ottolenghi's			=	No growth.

TABLE X.—*Same arrangement as Table IX. Plates inoculated six hours and eighteen hours after incubation*—Continued.

EIGHTEEN HOURS' INCUBATION.

Enrichment.			Growth on plates. Lactose agar.	Remarks.
Medium.	Reaction.	Concen-tration.		
		Per cent.		
Peptone	+1		—	Red colonies exclusively.
Do	+0.5		+	More than a few cholera colonies; red colonies predominate.
Do	—0.5		+	More than a few cholera colonies; same number of red colonies.
Do	—1		+	Numerous cholera colonies; more than a few red colonies.
Do	—2		+	More than a few cholera colonies; few small white colonies; cholera predom-inate.
Kraus's		20	—	Few small white colonies; no cholera.
Do		15	—	Same as 20 per cent.
Do		5	+	Numerous cholera colonies, pure.
Ottolenghi's			=	No growth.

TABLE XI.—*Same arrangement as Table X. Soda medium included. Plate inoculated after six hours' and twenty-four hours' incubation.*

SIX HOURS' INCUBATION.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reac-tion.	Con-centra-tion.	Lactose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+1		—		Numerous red colonies; no cholera.
Do	+0.5		—		Do.
Do	—0.5		—		Numerous red colonies; cholera, plus numerous small blue fluorescent colonies, blue ones predominate; no cholera.
Do	—1		—		Do.
Do	—2		+		Few cholera colonies; few red ones; numerous small blue fluorescent colonies.
Ottolenghi's			—		Few small yellow colonies.
Kraus's		10	+		Numerous cholera colonies; numerous small blue fluorescent colonies. Lat-ter predominate, no red ones.
Do		5	+		Numerous cholera colonies; numerous small blue fluorescent colonies. Lat-ter predominate.
Soda peptone	—1		+		Numerous cholera colonies; very few white colonies.
Do	—2		+		More than a few cholera colonies; one white colony.
Do	—3		+		Few cholera colonies; more than a few small white colonies.

TABLE XI.—*Same arrangement as Table X. Soda medium included. Plate inoculated after six hours' and twenty-four hours' incubation*—Contd.

TWENTY-FOUR HOURS' INCUBATION.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	+1		—		Red colonies exclusively.
Do	+0.5		0		
Do	—0.5		0		
Do	—1				Numerous cholera colonies; few red ones; few small fluorescent colonies.
Do	—2				More than a few cholera colonies; few red ones; few small fluorescent colonies.
Ottolenghi's					Small yellow colonies; few *subtilis*-like colonies.
Kraus's		10			Few cholera colonies; small blue fluorescent colonies predominate; very few red ones.
Do		5			More than a few small blue fluorescent colonies.
Soda peptone	—1				More than a few cholera colonies; few small white colonies and small blue fluorescent colonies; cholera predominate.
Do	—2		=		No growth.
Do	—3		=		Do.
Peptone	+1			=	Do.
Do	+0.5			+	More than a few cholera colonies.
Do	—0.5			+	Numerous cholera colonies, pure.
Do	—1			+	Do.
Do	—2			+	Do.
Ottolenghi's				=	No growth.
Kraus's		10		+	Numerous cholera colonies, pure.
Do		5		+	Do.
Soda peptone	—1			+	Very numerous cholera colonies, pure.
Do	—2			=	No growth.
Do	—3			=	Do.

TABLE XII.—*Same arrangement as Table XI. Incubation, six and twenty-four hours.*

SIX HOURS' INCUBATION.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	+1				Few cholera colonies; numerous blue spreading colonies; numerous red colonies.
Do	+0.5				The same as above, but colonies more numerous.

TABLE XII.—*Same arrangement as Table XI. Incubation, six and twenty-four hours*—Continued.

SIX HOURS' INCUBATION—Continued.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	−0.5		+		Numerous cholera colonies; numerous blue spreading colonies; the latter predominate; few red colonies.
Do	−1		+		The same as above, but colonies not so numerous.
Do	−2		+		More than a few cholera colonies; very numerous blue spreading colonies; a few red colonies.
Ottolenghi's			−		Two small white colonies; no cholera.
Kraus's		10	+		More than a few cholera colonies; few red ones; numerous blue spreading colonies.
Do		5	−		No cholera; numerous blue spreading colonies; few red colonies.
Soda peptone	−1		+		Numerous blue spreading colonies; numerous cholera colonies; cholera colonies predominate; few small white colonies.
Do	−2		+		Very few blue spreading colonies; more than a few small white colonies; few cholera colonies.
Do	−3		−		The same as above, but no cholera colonies.

TWENTY-FOUR HOURS' INCUBATION.

Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	Remarks.
Peptone	+1		−		Few red colonies; few blue ones; one cholera.
Do	+0.5		−		Do.
Do	−0.5		+		The same as above, but cholera colonies numerous.
Do	−1		+		Numerous cholera colonies; very few small white colonies.
Do	−2		+		More than a few cholera colonies; very few small white colonies.
Ottolenghi's			+		More than a few cholera colonies; more than a few small blue fluorescent colonies; few small white colonies.
Kraus's		10	+		Same as bile.
Do		5	+		More than a few cholera colonies; same number of small white colonies.
Soda peptone	−1		+		More than a few cholera colonies; very few small white colonies.
Do	−2		−		Few small white colonies; no cholera.
Do	−3		=		No growth.
Peptone	+1		=		Do.

TABLE XII.—*Same arrangement as Table XI. Incubation, six and twenty-four hours*—Continued.

TWENTY-FOUR HOURS' INCUBATION—Continued.

| Enrichment. | | | Growth on plates. | | Remarks. |
Medium.	Reac-tion.	Con-centra-tion.	Lac-tose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+0.5			=	No growth.
Do	—0.5			+	Numerous cholera colonies, pure.
Do	—1			+	Do.
Do	—2			+	Do.
Ottolenghi's				+	Do.
Kraus's		10		+	Do.
Do		5		+	Do.
Soda peptone	—1			+	Do.
Do	—2			=	No growth.
Do	—3			=	Do.

TABLE XIII.—*Same arrangement as Table XII. Plates inoculated after six hours' and twenty-four hours' incubation.*

SIX HOURS' INCUBATION.

| Enrichment. | | | Growth on plates. | | Remarks. |
Medium.	Reac-tion.	Con-centra-tion.	Lac-tose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+1		—		No cholera; numerous small blue opalescent colonies; few white ones.
Do	+0.5		—		No cholera; very numerous small blue opalescent colonies; a number of white ones.
Do	—0.5		+		More than a few cholera colonies; numerous small blue opalescent colonies; very few white ones.
Do	—1		+		Few cholera colonies; more than a few blue opalescent colonies.
Do	—2		+		Numerous cholera colonies; numerous small opalescent colonies; cholera colonies predominate.
Ottolenghi's			—		No cholera; few white colonies.
Kraus's		10	+		Same as —2 plus small white colonies.
Do		5	+		Same as —1.
Soda peptone	—1		+		Same as —0.5.
Do	—2		+		Very few cholera colonies; more than a few blue spreading colonies; numerous small yellow colonies.
Do	—3		—		Few blue opalescent colonies; no cholera.

TABLE XIII.—*Same arrangement as Table XII. Plates inoculated after six hours' and twenty-four hours' incubation*—Continued.

TWENTY-FOUR HOURS' INCUBATION.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reac-tion.	Con-centra-tion.	Lac-tose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+1		—		No cholera; more than a few white colonies.
Do	+0.5		0		
Do	—0.5		0		
Do	—1		+		Very few small white colonies; numerous cholera colonies.
Do	—2		+		More than a few cholera colonies; more than a few small blue opalescent colonies.
Ottolenghi's			—		Few small white colonies.
Kraus's		10	+		Few cholera colonies; numerous small blue opalescent colonies.
Do		5	+		More than a few cholera colonies; numerous small blue opalescent colonies.
Soda peptone	—1		+		Same as 10 per cent.
Do	—2		+		Same as —1.
Do	—3		+		No cholera; few small white colonies.
Peptone	+1			=	No growth.
Do	+0.5			=	Do.
Do	—0.5			=	Do.
Do	—1			+	Numerous cholera colonies, pure.
Do	—2			+	More than a few cholera colonies.
Ottolenghi's				=	No growth.
Kraus's		10		+	Few cholera colonies, pure.
Do		5		+	Very numerous cholera colonies, pure.
Soda peptone	—1			+	Do.
Do	—2			+	Do.
Do	—3			=	No growth.

TABLE XIV.—*Same arrangement as Table XIII. Plates inoculated after six hours' and twenty-four hours' incubation.*

SIX HOURS' INCUBATION.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reac-tion.	Con-centra-tion.	Lac-tose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+1		—		No cholera colonies; numerous red colonies.
Do	+0.5		—		Do.
Do	—0.5		+		Numerous cholera colonies; numerous blue spreading colonies; cholera predominates.
Do	—1		+		Same as —0.5, only cholera more numerous.

TABLE XIV.—*Same arrangement as Table XIII. Plates inoculated after six hours' and twenty-four hours' incubation*—Continued.

SIX HOURS' INCUBATION—Continued.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Peptone	—2		+		Cholera colonies numerous; few blue spreading colonies.
Ottolenghi's			—		No cholera colonies; few small white colonies.
Kraus's		10	+		Same as —0.5.
Do		5	+		Very few cholera colonies; numerous blue spreading colonies; numerous red colonies.
Soda peptone	—1		+		Numerous cholera colonies; a few blue spreading colonies; very few white colonies.
Do	—2		+		Very numerous cholera colonies; very few white colonies.
Do	—3		+		More than a few cholera colonies, pure.

TWENTY-FOUR HOURS' INCUBATION.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
Peptone	+1		—		No cholera colonies; a few red colonies.
Do	+0.5				
Do	—0.5				
Do	—1		+		Very numerous cholera colonies; very few red ones.
Do	—2		+		More than a few cholera colonies; very few red colonies.
Ottolenghi's			—		Few small white colonies; no cholera.
Kraus's		10	+		More than a few cholera colonies; numerous small blue opalescent colonies; very few red ones.
Do		5	+		Same as 10 per cent.
Soda peptone	—1		+		More than a few cholera colonies; very few small white colonies.
Do	—2		+		Do.
Do	—3		=		No growth.
Peptone	+1			=	Do.
Do	+0.5			+	More than a few cholera colonies, pure.
Do	—0.5			+	Numerous cholera colonies, pure.
Do	—1			+	Do.
Do	—2			+	Do.
Ottolenghi's				=	No growth.
Kraus's		10		+	Numerous cholera colonies, pure.
Do		5		+	Do.
Soda peptone	—1			+	More than a few cholera colonies, pure.
Do	—2			+	Do.
Do	—3			=	No growth.

TABLE XV.—*Same arrangement as Table XIV.* ·*Eighteen hours' incubation.*

Enrichment.			Growth on plates.		Remarks.
Medium.	Reac-tion.	Con-centra-tion.	Lac-tose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+1				Few red colonies; no cholera.
Do	−1				More than a few cholera colonies; more than a few red ones; cholera predominates.
Do	−2				Few cholera colonies; more than a few small white colonies; latter predominate.
Soda peptone	−1				More than a few cholera colonies; few small white ones; cholera predominate.
Do	−2				No growth.
Do	−3				Do.
Ottolenghi's					Few small white colonies.
Do		80			Do.
Do		60			Do.
Do		40			Do.
Do		20			Do.
Peptone	+1				No growth.
Do	−1				Very numerous cholera colonies, pure.
Do	−2				Numerous cholera colonies, pure.
Soda peptone	−1				Very numerous cholera colonies, pure.
Do	−2				No growth.
Do	−3				Do.
Ottolenghi's					Do.
Do		80			Do.
Do		60			Do.
Do		40			Do.
Do		20			Do.

TABLE XVI.—*Same arrangement as Table XI.* *Eighteen hours' incubation.*

Enrichment.			Growth on plates.		Remarks.
Medium.	Reac-tion.	Con-centra-tion.	Lac-tose agar.	Dieu-donné's agar.	
		Per ct.			
Peptone	+1		−		Few red colonies; no cholera.
Do	+0.5		+		Few cholera colonies; few red ones; latter predominate.
Do	−1		+		More than a few cholera colonies; few red ones.
Do	−2		+		More than a few cholera colonies; few spreading colonies; cholera predominate.
Ottolenghi's			−		Few small white colonies.
Kraus's		15	+		Numerous cholera colonies; few small white ones.

TABLE XVI.—*Same arrangement as Table XI. Eighteen hours' incuba-*
tion—Continued.

Enrichment.			Growth on plates.		Remarks.
Medium.	Reaction.	Concentration.	Lactose agar.	Dieudonné's agar.	
		Per ct.			
Kraus's		10	+		More than a few cholera colonies; equal number of red ones.
Do		5	+		Few cholera colonies; equal number of red ones.
Soda peptone	—1		+		More than a few cholera colonies; few white ones.
Do	—2		—		Few small white colonies; no cholera.
Do	—3		—		Do.
Peptone	+1			=	No growth.
Do	+0.5			+	More than a few cholera colonies, pure.
Do	—1			⊥	Do.
Do	—2			+	Numerous cholera colonies, pure.
Ottolenghi's				=	No growth.
Kraus's		15		+	Very numerous cholera colonies, pure.
Do		10		+	Do.
Do		5		+	More than a few cholera colonies, pure.
Soda peptone	—1			+	Numerous cholera, pure.
Do	—2			=	No growth.
Do	—3			=	Do.

SUMMARY

It will be seen from the protocols that the bacteria other than cholera vibrios, as encountered in cholera stools, can be divided into three groups from the practical standpoint, namely:

1. Bacteria which prefer an acid medium to an alkaline one; they are lactose fermenters—that is, representatives of the *coli* group. They were most frequently met with. Fortunately this class of bacteria can be successfully eliminated, or at least reduced so as not to interfere with the growth of the cholera vibrio, by alkaline reaction of the enrichment medium. They do not grow on Dieudonné's medium.

2. Bacteria which prefer strong alkaline reaction. They do not acidify lactose and are of little importance. Being cocci, they form small colonies, grow slowly, and occur in small numbers on the plates. They grow on Dieudonné's medium (Tables IV, VI, VIII, X, XI, XII, XIII, XIV, XV, XVI).

3. Bacteria which exhibit the same tolerance toward alkaline reaction of the medium as the cholera vibrio does and which have a broad range of growth in regard to the reaction of the medium. They were found to form numerous, some of them

spreading, colonies. Although the inhibition of growth by Dieu-donné's medium is evident in some cases, they do not grow on alkaline ox-blood agar (Tables II, VII, XI, XII, XIII). Bacteria of this class do not acidify lactose and are evidently dangerous competitors of the cholera vibrio during the enrichment process (Table II).

The number of examinations in which peptone solution of $+1$, $+0.5$, -0.5, and -1 reaction was used being about the same, the results allow a fair comparison. When $+1$ peptone was used, the cholera vibrio was recovered in 11 per cent; from peptone $+0.5$, reaction in 61 per cent; from -0.5 peptone solution, in 88.2 per cent; and peptone of -1 reaction gave 94.2 per cent positive results.

As to the bile medium the results were very unsatisfactory. It failed three times out of five. In our experiments the bile medium was prepared according to Ottolenghi's prescription, with the exception that dry bile was used instead of fresh, the latter not being available. Whether the low percentage of positive results was due to that fact or not I am not in a position to say. In this connection it may be of interest to mention that of the 20 strains of vibrios planted in human bile 2 strains of true cholera and 1 choleralike vibrio refused to grow in the bile.

Much better results were obtained with Kraus's medium. It was noticed that this medium eliminated the bacteria of the *coli* group more thoroughly than peptone solution, but the bacteria which prefer alkaline reaction thrive in it. Owing to the fact that the surface of the medium is of the least degree of alkalinity, they frequently form a pellicle, thus subduing the growth of the cholera vibrio. As the degree of alkaline reaction decreases with the age of the medium, more concentrated solutions of alkaline ox blood in meat broth are necessary in order to achieve the same selective effect.

All considered, Kraus's medium has a decided advantage over the peptone solution. The use of this medium will be particularly indicated in searching for carriers. In a laboratory like that of the Bureau of Science, where daily examinations of stools for cholera are being conducted all the year round, it is necessary that a sufficient amount of ox blood always be kept on hand, as Dieudonné's plates are indispensable in our work. This being the case, Kraus's medium can be easily prepared.

Combinations of peptone solution and selective enriching medium suggest themselves. The fact that the peptone solution

is more favorable for the rapid growth of the cholera vibrio, while the selective enriching medium inhibits the growth of the bacteria other than vibrios more thoroughly than the alkaline peptone solution, can be utilized in the double enrichment process with advantage in certain instances.

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I. EXPERIMENTS ON THE IMMUNIZATION OF GUINEA PIGS BY THE INOCULATION OF AVIRULENT TUBERCLE BACILLI IN AGAR. II. OBSERVATIONS ON ANIMALS INOCULATED WITH TUBERCULOSIS FROM LEPERS [1]

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In previous experiments [2] I succeeded in immunizing guinea pigs against virulent plague by the inoculation of small doses of living avirulent plague bacilli mixed with agar. These experiments have shown that the agar masses may persist for some time (twenty-nine days in one case), and that the plague bacilli multiply freely in such masses injected subcutaneously. In the following experiments living avirulent bacilli of tuberculosis were mixed with glycerin agar and inoculated. It was hoped by this method to obtain in tuberculosis a more effective immunization through the relatively slow absorption of a dose, comparatively small at the beginning and gradually increasing during absorption.

The avirulent strain of tuberculosis used was a human strain ("ki"), kindly furnished me by Dr. E. R. Baldwin of Saranac Laboratory, Saranac Lake, N. Y. It has been long cultivated in the laboratory, grows rapidly on glycerin agar, and is of very low virulence. In addition, a few inoculations were made with an avian strain.

Series 1, Table I.—In this short preliminary series (series I) the interval of time—one hundred sixty-eight days—between the immunizing and the virulent, or test, doses was relatively long. Only one immunizing dose was given, and that was relatively small. Some of the animals received avirulent human bacilli, mixed with agar, some received avian bacilli, and one animal received an emulsion of the avian strain without agar.

The avian dose was prepared by mixing about 30 cubic centimeters of 5 per cent glycerin agar with 3 cubic centimeters of a thick emulsion in salt solution of bacilli from glycerin agar culture. The dose of human bacilli was made in the same

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way, except that a somewhat smaller proportion of bacteria
was added to the agar.   The agar was cooled to about 40° before
adding the bacteria and was inoculated while still liquid.   All
inoculations were subcutaneous.   The needle was introduced
well into the subcutaneous tissue, since, if the agar is deposited
just under the skin, necrosis is likely to take place over it.   Care-
ful asepsis in inoculation was observed to avoid the introduction
of contaminating bacteria with the agar.   In addition to the agar
mixtures of both strains ("Tbag A" and "Tbag H" in the
tables), one animal was given a thick emulsion of the avian type
without agar.   Three cubic centimeters of the agar mixture
were given in all except one, which received 2.5 cubic centimeters.

Avian 6032 developed some diarrhœal disease with par-
alysis of the hind legs and was sacrificed eight days after in-
oculation.   Transfers from the agar lump in this animal to
nutrient medium showed a good growth of tuberculosis in pure
culture.   The other animals showed more or less infiltration
around the agar mass, which in some cases became the site of
an abscess with creamy pus.   Long before the virulent dose
was given, practically all lesions had disappeared except in
avian 6035, which had a lump about the size of a filbert at the
point of inoculation.   All animals were healthy, and six of the
nine had gained in weight.

The dose of virulent tuberculosis was given one hundred
sixty-eight days after the avirulent.   The dose was prepared
as follows: The sputum of three patients with pulmonary tuber-
culosis was mixed, making in all about 140 cubic centimeters.
The three samples examined microscopically showed in the first
moderately numerous tubercle bacilli, in the second many, and
in the third very many bacilli.   To the mixed sputum enough
antiformin was added to make a 25 per cent solution, and the
mixture was allowed to stand for half an hour to emulsify.
It was then centrifugalized at high speed.   The sediment was
washed with sterile distilled water and again with sterile salt
solution.   Microscopical examination showed a large number
of tubercle bacilli in this mixture.   All animals, immunized and
controls, were inoculated subcutaneously in the right inguinal
region with 0.5 cubic centimeter of this sediment, diluted with
a small quantity of salt solution.

The results are given in Table I.   The symbols +, ++,
+++ described roughly the size and number of palpable tuber-
cles formed in the inguinal region.   The first class includes
tubercles of approximately pea or small bean size, the second
of lima bean or hazelnut size, and the third larger sizes.

The results of all series are fairly uniform, and the discussion of this, as of Tables II, III, and IV, will be found after Table IV.

TABLE I.—*Series 1.　Comparatively small avirulent dose, part avian, part human.*

Guinea pig No.	Virulent dose, Dec. 20, 1911.			Weight, June 5, 1912. Inoculated with virulent Tb.	Aug. 6, 1912; 16 days after virulent dose.		Sept. 23, 1912; 110 days after virulent dose.	
	Strain.	Volume.	Weight.		Inguinal glands.	Weight.	Inguinal glands.	Weight.
		cc.	g.	g.		g.		g.
6030	Tbag A a	3	400	460	+	590	---------	---------
6031	----do	3	460	610	+	640	++	640
6032	----do	3	640	---------	---------	---------	---------	---------
6033	----do	2.5	490	500	+	480	+	410
6034	Emuls avian only	1	390	560	+	610	+	670
6035	Tbag H a	3	450	650	+	690	+	710
6036	----do	3	660	630	+	650	++	660
6037	----do	3	420	410	+	480	+++	520
6038	----do	3	500	410	-+	450	+	450
6039	----do	3	670	670	+	720	++	730
	Average	---------	---------	544.4	---------	590	---------	---------

Guinea pig No.	Nov. 16, 1912; 164 days after virulent dose.		Dec. 19, 1912; 197 days after virulent dose.		Died after virulent dose.	Autopsy.			
	Inguinal glands.	Weight.	Inguinal glands.	Weight.		Body weight.	Spleen.	Lesions.	Ratio of spleen weight to body weight.
		g.	—	g.	Days.	g.	g.		
6030	---------	---------	---------	---------	88	360	0.6	Tb.	0.0016
6031	++	530	---------	---------	179	510	4.2	Tb.	0.0082
6032	---------	---------	---------	---------	8	---------	---------	(b)	---------
6033	---------	---------	---------	---------	133	320	2.5	Tb.	0.0078
6034	+	680	+	640	349	480	3.5	Tb.	0.0073
6035	++	690	++	740	377	580	1.6	Tb.	0.0027
6036	++	650	++	640	311	470	2.6	Tb.	0.0055
6037	+++	470	+++	420	222	370	3.5	Tb.	0.0095
6038	+	410	---------	---------	187	340	5.4	Tb.	0.0159
6039	++	740	++	730	283	620	2.0	Tb.	0.0032
	---------	---------	---------	---------	236.6	---------	---------	---------	0.0069

a Tbag A and Tbag H refer to mixtures of agar with avian and human types of bacilli, respectively.
b Sacrificed.

Series 2, Table II.—In this series one hundred twenty-seven days intervened between the immunizing and the avirulent doses. For immunization only the human avirulent strain was used, and the animals were divided into four groups. The first group, "Tbag a," received an agar mixture prepared as follows: Three cubic centimeters of a thick emulsion from a 40-day culture was

TABLE II.—*Series 2. Animals receiving larger doses.*

Guinea pig No.	Avirulent dose. Jan. 30, 1912.			Apr. 29, 1912; 90 days after avirulent dose.		Inoculation of virulent Tb. June 5, 1912.	Aug. 6, 1912; 61 days after virulent dose.		Sept. 23, 1912; 110 days after virulent dose.	
	Strain.	Volume.	Weight.	Tumor.	Weight.	Weight.	Inguinal glands.	Weight.	Inguinal glands.	Weight.
		cc.	*g.*		*g.*	*g.*		*g.*		*g.*
6074	Tbag A ___	3	570	0	640	650	+	690	+ +	700
6075	___do ___	3	510	0	580	560	+	560	+	590
6076	___do ___	3	500	+	590	620	+	730	___	___
6077	___do ___	3	430	0	570	600	+	660	+	670
6078	___do ___	3	540	+ +	610	620	+	660	+	670
6079	___do ___	3	420	___	___	___				
6080	___do ___	3	390	+a	420	440	+	520	+	600
6081	___do ___	3	420	+a	530	550	+	630	+	640
6082	___do ___	2.5	430	+a	530	560	+	630	+	630
5889	Tbag B ___	1.5	410	0	590	600	+	610	+ +	630
5891	___do ___	3	540	+ +	620	640	+	680	+	700
5892	___do ___	3	560	0	740	740	+	750	+ +	790
6083	___do ___	3	470	0	570	620	+	650	+ +	660
6084	___do ___	3	400	+	500	500	+	550	+ +	560
6085	___do ___	3	340	0	450	490	+	530	+ +	550
6086	___do ___	3	300	0	370	390	+	460	+ +	530
5887	Tbag A ___	3	420	0	570	580	+ +	620	+ +	620
5888	___do ___	3	520	0	640	640	+	680	___	___
6043	___do ___	3	480	0	660	680	+	710	+ +	690
6087	___do ___	3	470	0	610	630	+	690	+ +	710
6088	___do ___	3	410	+ +	540	550	+	580	+	620
6089	Tb emuls A ___	0.5	580	+ +	610	610	+	670	+	730
6090	___do ___	1	510	0	580	590	−	660	+	670
6091	___do ___	0.3	400	0	510	510	+	560	+ +	570
6092	___ do ___	1	500	0	560	550	+?	610	+	640
6094	Tb emuls B ___	0.5	520	+a	580	560	+	620	+ +	620
6095	___do ___	0.5	430	0	540	560	+ +	640	+ +	630
6096	___do ___	0.5	440	___	___	___				
6097	___do ___	1	410	+	520	550	+	610	+ +	630
6098	___do ___	1	450	+	570	570	+?	___	___	___
	Average ___	___	___	___	___	573.2	___	___	___	___

a Slight.

TABLE II.—*Series 2. Animals receiving larger doses*—Continued.

Guinea pig No.	Nov. 16, 1912; 164 days after virulent dose.		Dec. 19, 1912; 197 days after virulent dose.		Died after virulent dose.	Autopsy.			
	Inguinal glands.	Weight.	Inguinal glands.	Weight.		Body weight.	Spleen.	Lesions.	Ratio of spleen weight to body weight.
		g.		*g.*	*Days.*	*g.*	*g.*		
6074	----------	----------		----------	160	700	7.1	Tb.	0.0101
6075	+	590	+	580	354	400	3.0	Tb.	0.0075
6076	----------		----------	----------				(a)	----------
6077	+	620	+	700	456	550	2.8	Tb.	0.0051
6078	+	680	+	720	812	500	1.0	Tb.	0.0020
6079	----------	----------	----------	----------	----------	420	----------	(b)	----------
6080	+ +	430	+	490	255	420	4.1	Tb.	0.0098
6081	+ +	670	+	690	319	560	4.5	Tb.	0.0080
6082	+ + +	600	+	620	240	570	4.3	Tb.	0.0075
5889	+ +	610	+	570	238	470	7.8	Tb.	0.0166
5891	+	690	+	710	418	600	3.0	Tb.	0.0050
5892	+	760	+ +	770	228	720	2.6	Tb.	0.0022
6083	+ + +	640	+ + +	650	313	580	2.4	Tb.	0.0041
6084	+	580	+	570	282	450	5.1	Tb.	0.0133
6085	+ +	490	----------	----------	203	430	3.3	Tb.	0.0077
6086	+	540	+	540	300	390	4.0	Tb.	0.0103
5887	+ +	610	+	600	227	490	2.9	Tb.	0.0059
5888	----------	----------	----------	----------	----------	----------	----------	(a)	----------
6043	+ + +	690	+ +	690	256	550	7.2	Tb.	0.0181
6087	+ +	700	+ +	740	380	610	1.5	Tb.	0.0024
6088	+	600	+ +	600	234	540	6.2	Tb.	0.0115
6089	+ +	730	+	750	345	400	3.2	Tb.	0.0080
6090	+ +	640	+ +	620	246	530	2.0	Tb.	0.0038
6091	+ +	570	+	560	304	490	4.0	Tb.	0.0082
6092	+ ?	690	+ ?	700	270	550	4.1	Tb.	0.0075
6094	+ +	630	+ +	630	315	490	9.2	Tb.	0.0188
6095	+ +	630	+ +	610	264	550	4.8	Tb.	0.0088
6096	----------	----------	----------	----------	----------	----------	----------	(c)	----------
6097	+ +	630	+ +	610	300	410	5.0	Tb.	0.0122
6098	----------	----------	----------	----------	----------	----------	----------	(a)	----------
	----------	----------	----------	----------	302.8	----------	----------	----------	0.0084

[a] Plague.   [b] Sacrificed.   [c] Intercurrent disease.

thoroughly mixed with 30 cubic centimeters of a 5 per cent glycerin agar made somewhat stiffer than usual. The culture had grown on 5 per cent glycerin agar to which a few drops of sterile unheated human serum had been added, and showed an abundant growth. Three cubic centimeters of this mixture were given to all animals except one, which received 2.25 cubic centimeters. The second group, "Tbag b," received the same agar mixture as the first group, except that the proportion of bacilli in the agar was doubled.

The third and fourth groups include animals which received emulsions of bacilli without agar. The third group, "Tb emuls a," received the same thick emulsion, undiluted, as that used in making "Tbag b." The total number of bacilli received by animals of the third group was evidently much larger than that given in the agar doses. Avian 6090, for example, received twenty-two times as many bacilli as avian 6074. The fourth group, "Tb emuls b," received thick emulsion in salt solution of a 55-day glycerin agar culture of the avirulent strain.

All doses were inoculated subcutaneously in the right inguinal region. The volume of the dose in all groups is given in the tables.

All agar-inoculated animals of series 2 showed marked infiltration around the agar mass, and seventeen days after inoculation a lump of agar-plus tissue, the size of a hazelnut or larger, was present. As shown in Table II, few had any marked lesions ninety days after the avirulent inoculation. At the time of the inoculation of virulent bacilli all were apparently healthy and all had gained in weight.

The virulent dose was exactly the same as that given in series 1 and was given in the same way and at the same time. Avian 6079 was sacrificed, avian 6096 died of some intercurrent infection before receiving the virulent dose, and Nos. 6076, 5888, and 6098 died of accidental plague infection of rat-flea origin after receiving the virulent dose. All others survived the virulent dose for at least one hundred sixty days and at autopsy showed typical lesions of tuberculosis.

Series 3, Table III.—In series 3 the animals received two avirulent doses. The second was given fifty days after the first. In preparing the first dose, an emulsion of a 26-day culture and one of an 11-day culture of the avirulent human strain were mixed and added to a 5 per cent glycerin agar containing 2 per cent agar, in the proportion of 1 cubic centimeter of emulsion to 35 cubic centimeters of agar. One animal received a thin emulsion without agar.

Three animals (Nos. 5990, 5991, and 5992) received this dose intraperitoneally; animal 5989, both intraperitoneally and subcutaneously; and the rest, subcutaneously in the inguinal region. The local reactions following the first subcutaneous dose were much the same as in the other series—infiltration for a few days and a hard lump which persisted for ten days or more. None showed more than a scar forty-four days after inoculation. One animal, No. 5986, died of sepsis two days after inoculation.

The character of the second inoculation is the same as in series 2 and is shown in Table III, where the same symbols are used in describing the dose as in Table II. All received the agar mixture, all a dose of 3 cubic centimeters, and all were inoculated subcutaneously in the right inguinal region.

The reaction following the second avirulent dose was more marked than in animals of series 2 not previously treated, which received the same dose. The effects were more permanent also, as may be seen by comparing the results in the two series after ninety days (Table III). This more marked reaction was probably due to a sensitization resulting from the first dose. All were well and gaining in weight when the virulent dose was given.

The virulent dose of series 3 was of the same character and amount as in the other series and was inoculated on the same day, in this series one hundred twenty-seven days after the second avirulent dose and one hundred seventy-seven days after the first. Animal 5991 died of some intercurrent disease before receiving the second avirulent dose.

Twelve controls received the virulent dose at the same time as the animals in series 1, 2, and 3. The same dose of sputum bacilli was given to all. The immediate reaction following the test dose was small, and on the whole, slightly less than that of the treated animals. The control group is given in Table IV.

In Tables I to IV the entry "Tb" indicates that the animal showed lesions of tuberculosis at autopsy. These in general were most marked in the inguinal glands, spleen, liver, and lungs. Tubercles in the mesentery or kidneys were rarely shown in the gross examination. Lung lesions were almost constant, consisting usually of many consolidated areas, although no cavity formation occurred such as has been described by some authors for chronic tuberculosis in guinea pigs.

SUMMARY OF THE RESULTS GIVEN IN TABLES I, II, III, AND IV

With regard to a possible immunization of the animals as judged by the length of time of survival after receiving the

TABLE III.—*Series 3. Animals receiving 2 immunizing doses.*

Guinea pig No.	First avirulent dose. Dec. 11, 1911.			Second avirulent dose. Jan. 30, 1912.			Apr. 29, 1912; 90 days after 2d dose. Tumor at point of inoculation.	Inoculation of virulent Tb. June 5, 1912. Weight	Aug. 6, 1912; 61 days after virulent dose.	
	Strain.	Volume.	Weight	Strain.	cc.	Weight			Inguinal glands.	Weight
		cc.	*g.*			*g.*		*g.*		*g.*
5980	Tbag	3	350	Tbag A	3	420	+ +	520	+	510
5981	do	3	440	do	3	500	+	600	+	670
5982	do	3	410	Tbag B	3	400	+	460	+	380
5983	do	3	450	do	3	500	+ +	650	+	670
5984	do	3	660	Tbag A	3	470	+	560	+ +	580
5985	do	2	600	Tbag B	3	630	0	690	⊣	700
5986	do	2	630							
5987	do	2	410	Tbag A	3	360	+	430	+	530
5988	do	2	350	do	3	360	+	630	+	680
5989	do	3	600	do	3	630	+ +	670	+	690
5990 [a]	do	3	430	do	3	440	+ + +	540	+	
5991 [a]	do	3	360	do	3	460				
5992 [a]	do	3	570	do	3	580	0	680	+	700
5994	Emuls only	2	630	do	3	630	+	710	+ ?	760
Average								595.0		

Guinea pig No.	Sept. 23, 1912; 110 days after virulent dose.		Nov. 16, 1912; 164 days after virulent dose.		Dec. 19, 1912; 197 days after virulent dose.		Died after virulent dose.	Autopsy.			
	Inguinal glands.	Weight	Inguinal glands.	Weight	Inguinal glands.	Weight		Body weight.	Spleen.	Lesions.	Ratio of spleen weight to body weight.
		g.		*g.*		*g.*	*Days.*		*g.*		
5980	+	420					110	420	2.7	Tb.	0.0064
5981	+	690	+ +	690	+ +	690	239	580	4.0	Tb.	0.0069
5982							97		2.1	Tb.	
5983	+	690	+	670	+	710	842	500	1.0	Tb.	0.0020
5984	+ +	570	+ +	500			183	440	5.2	Tb.	0.0119
5985	+ +	730	+ +	680	+ +	610	212	530	7.0	Tb.	0.0132
5986										(b)	
5987	+	580	+	580	+	580	291	540	4.2	Tb.	0.0078
5988	+	670	+ +	670	+ ⊣	700	380	520	2.0	Tb.	0.0038
5989	+ +	690	+ + +	600			189	550	8.1	Tb.	0.0147
5990 [a]	+	620	+	590	+	610	271	550	2.0	Tb.	0.0036
5991 [a]										(c)	
5992 [a]	+	730	+	720	+	730	453	650	3.2	Tb.	0.0049
5994	+	730	+ +	700	+ +	710	248	650	2.7	Tb.	0.0041
							292.9				0.0072

[a] First dose intraperitoneal; all other doses subcutaneous.
[b] Died from sepsis 2 days after first inoculation.
[c] Intercurrent disease.

TABLE IV.—*Controls of series 1, 2, and 3.*

Guinea pig No.	Inoculation of virulent Tb. June 5, 1912. Weight.	Aug. 6, 1912; 61 days after virulent dose.		Sept. 23, 1912; 110 days after virulent dose.		Nov. 16, 1912; 164 days after virulent dose.		Dec. 19, 1912; 197 days after virulent dose.		Apr. 14, 1913; 313 days after virulent dose.		Died after virulent dose.	Autopsy.			Ratio of spleen weight to body weight.
		Inguinal glands.	Weight.	Inguinal glands.	Weight.	Inguinal glands.	Weight.	Inguinal glands.	Weight.	Inguinal glands.	Weight.		Body weight.	Spleen.	Lesions.	
	g.	.	g.		g.		g.		g.		g.	Days.	g.	g.		
6252	480	+	500	+	580	+	550	+	610	+	630	512	670	3.8	Tb.	0.0057
6253	480	+	540	+	590	+	540	+	590	+	550	361	470	1.7	Tb.	0.0036
6254	480	+	400	+	440	------	------	------	------	------	------	159	400	2.6	Tb.	0.0065
6255	610	+	600	+	650	+	600	+	640	+	630	460	440	1.8	Tb.	0.0041
6256	590	+	550	+	610	+	540	+	580	+	465	465	560	1.9	Tb.	0.0035
6257	500	+	550	+	560	+	520	+	540	+	600	497	450	1.0	Tb.	0.0022
6258	530	+	470	+	420	------	------	------	------	------	------	132	390	8.7	Tb.	C. 0223
6259	430	------	----	------	----	------	----	------	----	------	------		310	------	(a)	--------
6260	600	+	600	++	620	++	550	++	480	----	------	202	460	3.1	Tb.	0.0067
6261	580	++	570	+	600	+	670	+	600	+	650	482	500	7.5	Tb.	0.0150
6262	540	+	510	+	500	------	------	------	------	------	------	136	450	8.2	Tb.	0.0182
6263	440	------	----	------	----	------	----	------	----	------	------	5	----	------	(a)	--------
	539	------	----	------	----	------	----	------	----	------	---	340.6	----	4.03	----	0.0088

ᵃ Intercurrent disease.

virulent dose, it is noteworthy that avian 6078, series 2, Table II, survived the virulent dose eight hundred twelve days, or over two years and two months, and that avian 5983 survived eight hundred forty-two days, or over two years and three months. However, if we take the series as a whole, we find that the average number of days of survival of the controls (Table IV) is higher than that of any series of treated animals.

These averages, compared with the controls, are as follows:

TABLE V.—*Average survival of treated and of control guinea pigs.*

Series.	Table.	Animals.	Average survival.
			Days.
1	I	9	236.6
2	II	25	308.8
3	III	11	292.9
Controls	IV	10	340.6

A few animals died of plague of rat-flea origin. These and the animals which died of any other intercurrent disease are re-

corded in the tables, but are not included in the averages. Animals 6078 and 5983 are included in the averages of Tables II and III, respectively.

If we compare the several groups of series 2, Table II, we have:

TABLE VI.—*Average survival of guinea pigs inoculated with different strains of bacilli.*

Bacillus strain.	Animals.	Average survival.
		Days.
"Tb ag a"	11	335.7
"Tb ag b"	7	283.1
"Tb emuls a"	4	291.3
"Tb emuls b"	3	293.0

If we compare the average weights of controls with those of the treated animals, both taken at the same time of inoculating the virulent dose, we have, including only animals which subsequently died of tuberculosis:

TABLE VII.—*Average weights of treated and of control guinea pigs.*

Series.	Animals.	Average weight.
		Grams.
1	9	544.4
2	25	573.2
3	11	595.0
Controls	10	539.0

In view of these averages it is evident that the greater resistance of the controls was not due to a selection of larger animals.

Considering the averages of all series, it appears that the preliminary treatment with avirulent bacilli in agar has afforded no protection against a subsequent dose of virulent bacilli. If of any effect, it has apparently tended to diminish the resistance of the animals. The avirulent bacilli, without agar, has also failed to immunize, so far as can be judged by the comparatively small number of animals in this series.

In the case of the two animals which survived the virulent dose over two years, however, there is some evidence of partial immunization. Their weights taken at the time of the inocula-

tion of the virulent dose were 620 grams for animal 6078 and 650 grams for animal 5983—weights greater than those of any control and greater than the average of any series; but that of animal 6078 was equalled or exceeded by five animals of the same series, and that of 5983 by four animals of its series. The change in weight of these animals is shown in the tables up to April 14, 1913.   Some later weighings are:

TABLE VIII.—*Animal No. 6078*

Date.	Weight in grams.
July 11, 1913	690
October 1, 1913	720
March 17, 1914	750
June 9, 1914	680
After death	500

Animal No. 5983.

July 11, 1913	710
October 1, 1913	710
March 17, 1914	740
June 9, 1914	670
After death	500

Both animals showed enlarged inguinal glands during the whole period following the virulent dose.   The fact that these enlargements appeared on both sides, increased at various periods, and persisted so long would make it unlikely that they were due to the avirulent inoculation alone and that neither guinea pig was infected by the virulent dose.   In animal 5983 inguinal abscesses formed and opened at least two years after the virulent dose.   In both animals the tumor formed at the point of inoculation by the last avirulent dose persisted for at least ninety days after that dose.   This greater reaction to the dose may have increased the amount of immunization.   The amount of this reaction, however, was equalled or exceeded by three other animals in each of the series to which animals 6078 and 5983 belong.   The average length of time of survival of the three of series 2 exhibiting the greater reaction was three hundred thirty-three days, only about twenty-one days above the average of the series, and the three of series 3 gave an average of only one hundred ninety days, considerably below that of the whole number in the series.   It is evident, then, that a greater reaction to the last immunizing dose was not necessarily followed by a greater resistance.

It is possible that these two animals exhibit only a greater natural resistance to infection.   One of the nontreated controls survived the virulent dose for five hundred twelve days.   However, the facts that animal 6078 survived this control by three

hundred days and that animal 5983 outlived it by three hundred thirty days afford an indication that the treated animals were in some degree immunized.

At autopsy animals 6078 and 5983 showed great emaciation with enlarged lymphatic glands and consolidated areas in the lungs. Sections of the lungs showed that these consolidated areas consisted mainly of fibrous tissue with very limited active processes. Tubercle bacilli were found in small numbers in the lungs of both and in the spleen of animal 6078 as well. Evidently a marked healing process was accompanying the progress of the tubercular lesions; but whether this healing was any more marked than in those nonimmunized controls which also exhibited a very chronic course of the disease is doubtful. On the whole, the evidence for immunization must rest largely on the longer survival of animals 6078 and 5983.

In summary, while there is some evidence of the partial immunization of these two animals, the average results of all animals give little encouragement for this method of treatment. It is possible that the method might be modified to serve a practical use in some immunization work—for example, that of cattle against bovine tuberculosis. The results obtained with the two animals long surviving indicate that the method is, at least, worth another trial in the same or a modified form.

A noteworthy fact in these experiments in both control and treated groups is the long life of a considerable number of animals after infection with the test dose and the steady gain in weight of some animals even for two hundred or three hundred days after becoming distinctly tuberculous. During the slow progress of the infection, glands often formed abscesses, which broke down and later healed, the animal continuing in comparatively good health.

In the explanation of the long survival of animals in the above groups four factors must be considered: namely, the condition under which the animals were kept, the size of the dose, the virulence of the dose, and the method of inoculation.

The conditions under which animals can be kept in the tropics differ widely, taken the whole season through, from those prevailing in most experiments on guinea pigs with tuberculosis conducted in northern countries. The temperature is fairly uniform, making it possible to maintain a good ventilation at all times. The animals were kept in a house closed on the sides by wire netting only, and were confined in roomy cages, which were open to ventilation on the top and sides. Except in the

few cases of females having young, only one animal was put
in a cage. They were given a uniform daily diet of cooked rice
and grass with no water except that contained in the rice.
Among the guinea pigs of this laboratory there have been few
of the epidemics not uncommon in many laboratories. Doubtless
these favorable conditions contributed to the resistance to tuber-
culosis of the animals used in these experiments.

Lack of virulence for guinea pigs in bacilli from the mixed
sputum of three human pulmonary cases would hardly be ex-
pected, and the short treatment with 25 per cent antiformin was
scarcely sufficient to affect the virulence. The size of the dose
could not be closely estimated since the proportion of dead bacilli
in the sputum could not be known. Nearly all animals, however,
showed tubercles within a short time after inoculation. In the
subcutaneous inoculation a slower progress of the disease would
be expected than by the intraperitoneal.

In any case, either the smallness of the dose or a possible
lack of virulence must have affected the result, since animals
in other groups (see Tables V and VI), kept under the same
conditions and inoculated subcutaneously with bacilli from a pure
culture of another origin, survived a much shorter time.

OBSERVATION ON ANIMALS INOCULATED WITH TUBERCULOSIS FROM LEPERS

SERIES A

On October 9, 1911, a monkey was inoculated subcutaneously
with spleen pulp taken at post mortem from a case of leprosy
which showed very numerous leprosy bacilli in the spleen. This
monkey (No. 5804) died December 6, 1911, with lesions of
tuberculosis. Spleen emulsion from monkey 5804 was inoculated
into monkey 5975, which died twenty-nine days later (primarily
of tuberculosis). From the inguinal glands of this monkey a
pure culture was made. This culture was inoculated May 31,
1912, into a series of 20 guinea pigs. An amulsion in salt
solution was made of a 106-day culture on glycerin agar plus a
few drops of human serum. A portion of this emulsion was
further diluted with salt solution, and a portion was mixed in a
stiff agar containing 5 per cent glycerin. Dilutions were made
so that the dose employed, 2 cubic centimeters, contained in both
salt solution and agar approximately the same quantity, about
0.04 of the original culture. By counting, the dose was found
roughly to approximate 1,000,000 bacilli. All inoculations were

made subcutaneously in the left inguinal region. The results are given in Table IX.

TABLE IX.—*Series A. Animals inoculated with a culture of tuberculosis of leper origin.*

Gui-nea pig No.	Weight	May 3, 1914. Dose, two cubic centimeters.	95 days after inoculation.		Died after inocu-lation.	Autopsy.			
			Tuber-cle.	Weight		Body weight.	Spleen.	Le-sions.	Ratio of spleen weight to body weight.
	g.			*g.*	*Days.*	*g.*	*g.*		
6204	500	Agar			29			Tb.	
6205	330	...do	+	400	173	300	6.3	Tb.	0.0210
6206	550	...do			91	500	8.6	Tb.	0.0172
6207	410	...do			60			Tb.	
6208	300	...do			74	280		Tb.	
6209	480	...do			2			(a)	
6210	300	...do	+	370	170	300	4.0	Tb.	0.0133
6211	530	...do			73	370		Tb.	
6212	390	...do	+	410	106	350	10.5	Tb.	0.0300
6213	340	...do	++	420	132	132	5.1	Tb.	0.0124
6214	440	...do	++	430	196		5.0	Tb.	
6215a	360	...do			78	300	3.7	Tb.	0.0123
6215b	400	...do	++	430	122	350	6.9	Tb.	0.0197
6216	500	...do	+++	390	184	838	0.7	Tb.	0.0021
6217	600	...do			27			(b)	
6218	330	Emuls	++	400	190	340	10.1	Tb.	0.0297
6219	490	...do			114	440	2.4	Tb.	0.0055
6220	340	...do	+	480	154	420	12.6	Tb.	0.0300
6221	420	...do	+	430	104	410	14.7	Tb.	0.0359
6222	520	Agar no Tb	0	550					
		Average			120.6		6.9		0.0191

[a] Sepsis.   [b] Intercurrent disease.

The average survival of the animals in this group is 120.6 days for the 17 dying of tuberculosis—a time much shorter than that of the animals in series 1, 2, and 3, inoculated with tubercle bacilli from sputum. The majority gained weight up to ninety-five days after inoculation, but all had died by the one hundred ninety-sixth day, and those receiving bacilli in agar died on the average sooner than those receiving the emulsion only, although the latter group was too small to form a basis for any general conclusion.

In order further to test this strain of tuberculosis, guinea pigs were inoculated with material from the much enlarged spleens of different animals of the above group which died of tuberculosis. All were inoculated subcutaneously and all with a small portion of the spleen pulp. The results are given in Table X.

TABLE X.—*Series A.   Animals inoculated with material from enlarged spleens.*

Guinea pig No.	Weight.	Material inoculated from spleen of—	Date of inoculation.	Weight.			Died after inoculation.	Autopsy.			
				Nov. 16, 1912.	Dec. 19, 1912.	Apr. 14, 1913.		Body weight.	Spleen.	Lesions.	Ratio of spleen weight to body weight.
	g.		1912.	*g.*	*g.*	*g.*	*Days.*	*g.*	*g.*		
5221	680	6221	Aug. 16	500	------	------	98	410	13.2	Tb.	0.0328
5216	460	6221	do	420	------	------	96	410	12.5	Tb.	0.0305
6400	500	6218	Sept. 7	470	480	------	193	380	------	Tb.	---------
6401	670	6218	do	570	590	------	201	510	2.0	Tb.	0.0039
6426	410	6220	Oct. 4	430	480	420	201	360	5.6	Tb.	0.0156
6427	450	6220	do	330	350	370	222	320	1.6	Tb.	0.0050
6428	450	6220	do	460	490	490	224	400	5.0	Tb.	0.0125
			Average	------	------	------	176.4	------	6.6	------	0.0167

A striking result in the autopsy findings in series A is the unusual enlargement of the spleen.   This was shown in both culture-inoculated and spleen-inoculated groups.   The weight of the spleen at autopsy showed the very high maximum of 14.7 grams to 410 grams body weight in animal 6221, and the high average of 6.9 grams for all of series A of which spleen weights were taken.

As a basis of comparison the ratio of the spleen weight to the body weight at autopsy was calculated for a considerable number of the guinea pigs dying of tuberculosis of human-sputum origin. These animals belong to series 1, 2, and 3, treated animals of part I of this paper, and the controls of that series.   The averages of these ratios compared with those of leper series B are given in Table XI.

TABLE XI.—*Average ratios of spleen weights to body weights in all series.*

Series.	Table No.	Origin of tubercle bacilli inoculated.	Average of ratios, spleen weight to body weight.	Average survival after inoculation.	Animals averaged.
				Days.	
1	I	Sputum	0.0069	236.6	9
2	II	do	0.0084	308.8	25
3	III	do	0.0072	310.7	11
Controls	IV	do	0.0088	340.6	10
A	V	Leper spleen	0.0191	134.6	12
A	VI	do	0.0167	173.7	6

It will be seen in Table XI that the average ratios of the two leper series far exceeds that of any sputum series; in fact, that of A, Table X, the lowest of the two leper series, is nearly double the highest of the other series.

The average number of days of survival of the leper series is much below that of any sputum series; but by comparing individual ratios in all tables with the corresponding number of days of survival, it does not appear that there is any constant correlation between the time of survival and the enlargement of the spleen. The evidence is good that we have to do with a strain of tuberculosis which in guinea pigs tends to enlarge the spleen to a greater degree than occurred in the other series of animals inoculated with the mixed strains from sputum. In calculating the average number of days of survival in Table VII, only those animals are included of which the spleen weight was known.

SERIES B

Spleen pulp from a human case of leprosy, taken at post mortem, was inoculated August 5, 1912, into four guinea pigs subcutaneously. The leprosy case was well advanced and showed numerous lepra bacilli in the spleen pulp and glands. Two of the four guinea pigs developed tubercles in the inguinal region in less than forty days after inoculation, while the other two showed no signs of infection after having been kept under observation over one and one-half years. One, 6349, died November 7, 1913, about one year and three months after inoculation, with numerous tubercles in the spleen, liver, and lungs, and enormous numbers of acid-fast bacilli in the liver. The spleen weight was 2.1 grams; its ratio to body weight, 0.0051. A portion of the spleen pulp of 6347 was inoculated into a new guinea pig, 6816. This guinea pig died two hundred twelve days after inoculation, showing tubercles in spleen, liver, and inguinal glands. The ratio of spleen to body weight in this case was 0.0084. On June 8, 1914, a mixture of spleen and liver tissue was inoculated subcutaneously into guinea pigs 6970 and 6971. These, at present, October 10, 1914, exhibit palpable tubercles in the inguinal region.

SERIES C

Two guinea pigs were inoculated with the spleen pulp of a third human case of leprosy on August 14, 1912; no acid-fast bacilli were found in a smear from the spleen. After over two years of observation, no signs of tuberculosis has appeared in either of these animals.

SERIES D

Five guinea pigs were inoculated August 16, 1913, with spleen pulp of a case of human leprosy. Acid-fast bacilli were not found in the human spleen. One animal died of sepsis soon after inoculation. None of the other four developed any signs of tuberculosis.

SERIES E

Five guinea pigs were inoculated August 18, 1913, with spleen pulp of a case of leprosy, tubercular form. Numerous bacilli lepræ were found in the spleen. Of these, three animals show no signs of tuberculosis after over one year's observation. One died about one year after inoculation with no signs of tuberculosis, and one died forty-two days after inoculation, with enlarged inguinal glands, and apparently tubercles in the lungs and spleen, but acid-fact bacilli were not found in smears from the inguinal glands. Probably this animal died of some other disease.

SUMMARY

1. Five series of guinea pigs or monkeys were inoculated with the spleen pulp of lepers taken at post mortem. Lesions of tuberculosis or lesions very similar to those of tuberculosis developed in two of these series. In one of the two series only part of the pigs developed tuberculosis. In one (series E) one guinea pig out of five showed lesions, possibly those of tuberculosis.

2. A series of guinea pigs inoculated with a strain of tuberculosis of leper-spleen origin (series A) exhibited at post mortem a remarkable enlargement of the spleen. The average ratios of the spleen weight to the body weight at post mortem were nearly double the average ratios of a series dying of tuberculosis of human-sputum origin.

A TEST OF COCCOBACILLUS ACRIDIORUM D'HERELLE ON LOCUSTS IN THE PHILIPPINES [1]

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In view of the reported success following the use of *Coccobacillus acridiorum* d'Herelle in the destruction of locusts in Argentina, South America, it was deemed advisable to test the method in the Philippine Islands.

It may be stated at the outset that we are unable to obtain any results of practical value, but in view of the number of experiments made and the thoroughness of the trial, it has seemed worth while to record our negative results.

A sealed agar culture, arriving in perfect condition, was received in May, 1913. It bore the label of the Pasteur Institute, and complete directions for its use were sent with it. Following the directions, we proceeded to exalt the virulence of the culture and to inoculate a series of locusts with a broth culture of the bacillus. Subsequent lots were inoculated with material taken from dead or dying insects of the preceding series, and so on. The locusts chosen were adults, fairly fresh from the field, and the inoculated ones and controls were kept in large cages and were supplied with food. A layer of white filter paper on the bottom of each cage served to reveal the presence of diarrhœal fæces. In inoculation a fine-pointed glass pipette was used instead of a syringe, since the former appeared more convenient to handle and allowed a more accurate dosage. The technique followed was a modified form of a technique used by one of us [2] in the inoculation of cockroaches with plague bacilli. Inoculations were made into the abdomen as directed, and dilutions were made with broth in later transfers. Practically every dose was examined microscopically, and cultures were made at each inoculation.

Several exaltation series were carried out. In one, carried to the thirtieth set of insects, we used for the most part diluted

fæces for inoculation, pressing it out of the abdomen and diluting with broth as the directions recommended. For convenience in reference we have designated this series as 30x (Table I).

We early found that our inoculated insects did not show the one characteristic described—the liquid excrement. This may have been present in a few cases, but was decidedly rare. Since we often failed to find fæces with the abundant actively motile bacilli as described in the directions, we did not always dilute so highly with broth as the author of the directions recommends. It was found that if we gave too light a dose, even after a considerable number of insect transfers, the death of the insects was long delayed. The volume of the dose as given in the directions, "two or three drops," is rather indefinite, and we may have averaged a somewhat smaller volume of material. But since the effective dose depends on the number of viable bacteria, this ought not to make a material difference, especially in view of the fact that increase of virulence of the bacteria should go on as well with small doses as with large, provided only that enough is given to cause a fatal infection.

On account of the inconstancy of the number and character of the bacteria in the fæces, we undertook a second exaltation series carried out in another way. Here any material from the gut was carefully avoided, and the bacilli were taken wholly from the body cavity. This was accomplished in most inoculations by injecting broth into the body cavity of the dead or moribund insect, withdrawing it by means of the pipette, and using this liquid for inoculation—always after microscopical examination to assure us of the presence of motile organisms. This series, carried through 15 insect transfers, we have designated as 15x (Table II).

Later, when field experiments with 30x and 15x had given no practical results, a third exaltation series was carried out. In this series the inoculation material consisted of fluid pressed with aseptic precautions from the leg of a dead or moribund insect. When full of actively motile coccoid forms, as was usually the case, this liquid was diluted with sterile broth and used for the next set. Wingless locusts in the later stage were used for the most part, and the series was carried to the twelfth insect transfer. This series we have designated 12x.

In the first two series especially, we often divided the lot of insects to be inoculated at any one time into several sets, one set receiving material from the gut, another from the body cavity of the dead insect used as a source of material, or one lot was

given a larger and another set a smaller dose. This gave us some criterion of the amount and character of dosage to use. In each of the three series of insects inoculated there were lots in which some or all of the locusts inoculated with moderate doses died within from six to eight hours after inoculation; so that we had apparently reached the degree of virulence required by the directions.

In each of the three series the starting culture was that received from the original source. In order to make sure that the culture which passed through a series was the same as that used in starting, a careful comparison was made of the culture obtained from insects at the end of the 12x series with that used at the beginning. Both were found to have the same appearance and motility in hanging drop, and both were Gram-negative and exhibited the same morphology in stained specimens. Both showed the same rapid growth in plain agar, and agreed in showing very slight gas, with little or no acid, in lactose litmus agar and in lactose broth fermentation tubes. In glucose broth fermentation tubes both formed gas to the extent of about seven tenths of the volume of the closed tube, and both showed gas and acid in maltose litmus agar and in mannite litmus agar. Neither showed gas nor acid in saccharose litmus agar. It is possible that these sugars were not pure in every case, since they had been kept for some time in the tropics; but however that may have been, it is to the last degree unlikely that a contaminating organism would show so many characteristics in common with the original culture. In one of the control series (see below), *Bacillus prodigiosus* was used for a series in place of *Coccobacillus acridiorum*. This easily recognized organism was recovered from the body of an insect after the twelfth insect transfer.

As controls, material was taken from the body contents of 10 healthy locusts taken directly from the field and was spread on agar in test tubes. Nine of these tests showed no growth, while one exhibited 3 colonies, possibly contaminants. The method of making these cultures as well as of taking cultures from infected insects was as follows: The posterior leg of a locust was removed, preferably above the trochantofemoral joint. The distal part of the femur was held between the thumb and finger, and alcohol was dripped over it in order partially to sterilize the surface. After the alcohol became dry, the end of the femur was cut off with hot scissors, and some of the contents of the leg were pressed upward until they appeared

at the cut surface.   They were then touched with the sterile loop and transferred to an agar slope.   Abundant growth practically never failed in the test tube when microscopical examination had previously shown the presence of bacteria in the body cavity.

CONTROLS

During the exaltation series, controls of uninoculated insects were kept; and besides, some insects were inoculated with broth alone.   Such controls remained in good condition for days with but little diminished numbers.   In addition to the above, controls were made of insects inoculated with other bacteria.   *Bacillus prodigiosus* was carried through 12 insects transfers at the same time as 12x of *Coccobacillus acridiorum*.   The death of the insects followed the inoculation with about the same regularity and after as short an interval as in the case of *Coccobacillus*. Cultures were made from the insects after many passages and sprayed on the food of locusts in corrals and in the field.   Several insects found dead in the corrals showed *Bacillus prodigiosus* apparently in pure culture in the body cavity.   Precautions were taken to avoid surface and gut contamination in making cultures.   One insect found dead in the field after spraying with *Bacillus prodigiosus* also showed this organism in the body cavity.

Another control series was started with inoculations of the gut contents of an insect which died at a station some distance from the laboratory where inoculation experiments were being carried on.   At this station there was no possibility of accidental infection with *Coccobacillus acridiorum* from the laboratory. Insects died just as promptly after similar intraabdominal doses of this material as after doses of the *Coccobacillus*, and ingestion experiments in cages gave, if anything, better results (Tables III and V "Singalong").   Field experiments were alike negative with both strains.

A special experiment was arranged to compare the effect of small doses of the original culture of *Coccobacillus acridiorum*, as received, with those of a culture of the same source which had been passed through a series of locusts ("12x" series). The exalted culture had been passed through 12 series of locusts, with one or two exceptions in the nymph stage.   It was kept at refrigerator temperature for about three weeks, with the exception of about three days at room temperature.   This culture came directly from the leg of an infected insect.   It was then planted on agar to get a fresh growth and inoculated into a set of mature locusts.   From the first one dying, a new set

of mature locusts was inoculated, and from the first or second dying in this series, an agar culture was made from the leg. It had then been passed through 14 series of insects, the last two immediately before the experiment.

The stock culture had been transferred about four times on agar and kept at room temperature for about six weeks. A broth emulsion was made of a 10-hour agar culture of this stock strain and a similar emulsion of the "exalted" strain.

With a fine, very sharp capillary pipette, approximately equal doses of each emulsion were inoculated into the abdominal cavity of mature locusts recently taken from the field. The dose was gauged by a mark on the pipette, and the same pipette was used for all inoculations. It was sterilized in hot water after the inoculation of each lot. The size of the dose was larger, if there was any material difference, for the exalted strain than for the stock culture, and the exalted strain was inoculated after the stock, so that any growth taking place in the broth would tend to make the exalted strain larger. The aim was to have any error in the direction of increasing the exalted culture corrected. The dose of the exalted culture, as measured in the Thoma Zeiss counting chamber, approximated 2 cubic millimeters; and by measuring the dimensions of the lumen of the capillary, approximately the same result was obtained. The number of bacteria per dose roughly approximated 1,500,000.

Fifty locusts were inoculated with each strain, and as controls, 50 were inoculated with the same dose of sterile broth and 50 were placed in a cage with no treatment.

The four lots were placed in separate cages under similar conditions. The results are given in Table I.

TABLE I.—*Locusts inoculated with Coccobacillus acridiorum.*

Lot No.	Dose, about 2 cubic milli-meters of—	Locusts inoculated.	Locusts dead or moribund at the end of—								
			Hours.			Days.					
			22.	25.	34.	2.	3.	4.	5.	7.	17.
1	Stock culture	50	1	1	2	21	34	40	41	42	a 47
2	Exalted strain, 14 insect passages	50	1	5	21	34	44	46	a 47		
3	Broth alone	50		1	1	3	6	7	7	7	12
4	No inoculation	50		1	1	2	4	5	5	5	9

a All dead.

The table shows that the insects died somewhat sooner from the dose of the exalted strain than from that of the stock

culture. The exalted dose may have been slightly larger, but it is not probable that this excess alone could account for the difference. The slow response to the inoculation in both series was probably due to the small size of the dose. A few locusts escaped from the cages, and although the dead insects were removed at each examination, some may have been eaten by others; so that the total number at the close does not reach quite 50.

The white filter paper covering the bottoms of the cages showed few traces of diarrhœa in either, and less in the cage containing those inoculated with the exalted strain than in the other.

Ingestion experiments were conducted in relatively small cages in the laboratory, in similar cages placed on the grass of a lawn, in corrals made of galvanized iron, and in the open field on a large scale. The results of the field experiments are given in Table II.

By far the most attention was given to the field experiments. The insects here were for the most part wingless and varied from nymphs soon after emerging from the egg, in one series, to nymphs of the third to fifth instar. The greater number of tests were made on insects of the latter size. Automatic sprayers of a good type ("Autospray" No. 1, Rochester, N. Y.) were used, and the infective material was sprayed as early in the day as the insects began to feed well. The material was sprayed on the grass or other food in, and just in front of, the advancing swarm.

Shipments were received from the laboratory daily of a number of large bottles containing sterile broth. Usually two such bottles, each containing from 2.5 to 3 liters of broth, were used for a single spraying. Broth cultures started the day before were used, and never until they had become well clouded. A much larger amount of broth culture was used in proportion to the area than that recommended by the directions.

The experiments extended over a period of more than twenty days, and through one period of wet weather, although for the most part the weather was hot and dry. The material sprayed consisted of cultures of 30x, 15x, 12x, and the control strains, *Bacillus prodigiosus* and Singalong. In the case of the strains exalted in the laboratory, 30x, 15x, and Singalong cultures were taken directly to the field, about half a day's journey from the laboratory, immediately after the last insect transfer, and the stock cultures were kept in a refrigerator in the field. In the case of 12x, the strain exalted during the field exper-

TABLE II.—*Field experiments with Coccobacillus acridiorum at Cabanatuan.*

Date.	Condition of field.	Age of locust.	Culture sprayed.	Time sprayed. A. M.	Time sprayed. P. M.	Weather conditions.	Results.
1913.							
June 3	Rice paddy, very dry	Nymphs. Third instar.	Twelve hours' broth cultures 30x and XIX of Singalong.	6.15	5.00	Hot and very dry	Negative.
June 4	High cogon, river bottom sand, moist.	Nymphs. First and second instar.	Twelve hours' broth 30x plain and with 10 per cent solution molasses and stale beer.	6.00		Hot and dry	Do.
June 5	Rice paddy, very wet.	Nymphs. Third instar.	Twelve hours' broth 30x, Singalong, and 15x	9.15		Relatively cool and damp.	Do.
June 6	---do---	---do---	Twelve hours' broth 30x and crushed hoppers	7.30		Warm and damp	Partially negative.
June 7	---do---	---do---	Twelve hours' broth 30x	9.15		---do---	Negative.
June 8	---do---	Nymphs. Fourth instar.	30x, 15x, and 21 Singalong, crushed hoppers and 20 per cent stale beer and molasses.	9.00		---do---	Do.
June 9	---do---	---do---	Crushed hoppers 30x and 15x and 21 Singalong.	9.15		---do---	Do.
June 10	---do---	---do---	---do---	9.30		---do---	Do.
June 11	---do---	---do---	---do---	(a)		---do---	Do.
June 12	River bottom, high cogon, sandy.	Third and fourth instar.	30x	10.00		Rainy and warm	Do.
June 13	---do---	---do---	30x and Singalong	9.30		Warm and damp	Do.
June 14	---do---	---do---	---do---	9.30		---do---	Do.
June 15	---do---	---do---	---do---	10.00		---do---	Do.
June 16	---do---	---do---	---do---	9.30		---do---	Do.
June 17	---do---	---do---	---do---	9.30		---do---	Do.
June 18	---do---	---do---	30x, Singalong, and *B. prodigiosus*	11.00		---do---	Do.
June 19	---do---	---do---	30x, 12x	10.00		---do---	Do.
June 20	---do---	---do---	30x, 12x, and *B. prodigiosus*, plus molasses and tiqui-tiqui.	11.00		---do---	Do.
June 21	---do---	---do---	---do---	10.00		---do---	Do.
June 22	---do---	---do---	---do---	10.00		---do---	Do.

[a] Intermittent, from 9 to 11 a. m.

iments, broth was inoculated from young cultures, and in some cases directly from the body cavity of infected insects. In one of our field-corral experiments a considerable number of insects died after inoculation with one of the exalted strains. These insects were collected, crushed in broth, and the broth, after two or three hours' growth, was sprayed in the open field. Our one partially positive field experiment followed a spraying of this material. We gave our personal attention to the spraying and examination of the swarms.

Since the insects usually did not begin to feed freely until the day was well advanced, it was thought that our negative results might be due to the fact that the insects did not ingest a sufficient quantity of culture before it had been killed or attenuated by the heat or dryness. So another method of feeding was employed in some later experiments. Fresh broth cultures were mixed with rice polishings (tiqui-tiqui) and a small amount of sirup immediately before being set out for the insects in the field. The insects fed on this mixture greedily, collecting on it immediately after it was exposed. Two such experiments gave results as clearly negative as the ordinary spraying. Intermittent spraying practiced on the same swarm during the course of a morning likewise gave negative results.

The fields were visited on the day following spraying and usually on several subsequent days, and careful search was made for dead or infected insects. As stated before, only one experiment gave partially positive results. This experiment was conducted in an open paddy field during a relatively wet period. The material sprayed was broth in which were crushed a large number of insects found dead in a corral experiment. A considerable number of insects, amounting perhaps to several liters, were found dead in the field the following day; although the dead were but a small percentage of the total swarm, and apparently there was no marked diminution of the swarm. There was absolutely no indication of a natural spread of the infection in this swarm, and cultures from dead insects found in the field and emulsions of the body contents of these insects failed to give positive results in subsequent sprayings. In no case was there any indication of the prevalence of diarrhœa among insects in the field.

We do not believe that our failure to obtain field results of practical value was due to any error in technique or any lack of thoroughness in the test. Two conditions may have contributed to make our results less successful than those reported from

Argentine—the species of insects and the prevailing high temperature.

Two species of locusts in mixed swarms were sprayed during these experiments; namely *Œdaleus nigrofasciatus* DeGeer, and *Locusta migratoroides* R. and F.[3] The room temperature ranged from 28° to 32° C. or over during the time of these experiments, and the temperature in the fields exposed to the sun must have been much higher. Even during the short rainy period (the time when we attained a partial success) the temperature, though somewhat lower, remained relatively high. The author of the directions especially cautions against cultivating the organism at ordinary incubator temperature. If high temperature is an obstacle to attaining or maintaining an effective virulence of the bacterium, the method is much handicapped in the Philippines, where the insects often pass through the nymph stage during the hot dry season.

In order to determine whether or not our failure to obtain practical results with *Coccobacillus acridiorum* d'Herelle might have been due to some error in technique, the Bureau of Science sent to M. d'Herelle, at the Pasteur Institute, a detailed report of our 1913 experiments and their results. The following is an extract (translated from the French) of the reply kindly transmitted to the Bureau by M. d'Herelle and received November 11, 1913:

> Your lack of success doubtless must be attributed to a lack of strength of the virulence. Dr. Sergent, director of the Pasteur Institute in Algiers, while working with *Stauronoutus maroccanus*, was obliged to obtain 56 passages before obtaining a coccobacillus sufficiently strong to propagate the epizoötic in the field. As the locust in the Philippine Islands also belongs to a different genus from that of America, where the virus originated, doubtless the passages must be multiplied in order to adapt the microbe (for use in the field).

The directions accompanying the cultures stated that usually 12 passages suffice to exalt the virulence to the necessary degree, but that the final test of virulence is that the coccobacillus be sufficiently virulent to kill the inoculated locusts within from eight to ten hours. We carried one of our series to the thirtieth passage, another to the fifteenth, and a third to twelfth. In a series conducted in May, 1914, in Mindoro by one of us a series was carried to the twenty-third passage. Judging by the time necessary for the bacterium to kill the inoculated insect, we had in all of the series a virus of fully sufficient exaltation. In reference to the experiments on the grasshopper

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[3] Identified by A. N. Condil, United States National Museum.

in Algeria it appears from reports received from the American Consul at Algiers (see page 175) that no satisfactory field results were obtained even from a virus that had passed through from 74 to 87 grasshoppers. In laboratory experiments, however, many passages apparently were necessary, in the Algerian grasshoppers, to exalt the virus to a sufficient degree. According to the report mentioned above, the virus, at first not certainly fatal after a period of from twenty-four to thirty-six hours following artificial inoculation, was exalted to a degree where it was invariably fatal within four hours.

Experiments on locusts with *Coccobacillus acridiorum* were continued in May, 1914, in Mindoro by one of us (Barber). A new culture obtained from Argentina was used and submitted to 23 locust passages. In the laboratory experiments in this series there was more tendency among infected insects to discharge liquid excrement than was observed in the experiments of 1913 in Luzon, but this diarrhœa was by no means a constant symptom.

Field experiments were conducted with cultures from insects where the bacterial dose (diluted ten times) was sufficiently strong to kill inoculated insects within six hours.

In the field experiments infection was attempted on both winged locusts and "hoppers." The cultures were applied to the grass or cane on which the insects were feeding in several different ways: namely, spraying or broth cultures alone, broth cultures plus meal and molasses, and the extract from dead crushed insects. These were taken from a large cage, where they had been fed on a presumably exalted virus. Locusts confined in cages during this series of experiments fed much better than during our experiments of 1913. Grass soaked in culture media was devoured immediately, and healthy insects readily fed on the dead ones. So, in order to get a further method of spreading the infection in the field, locusts were caught, fed on culture-soaked grass, and turned loose among the field swarms.

In not a single instance during the whole of the Mindoro experiments was there the slightest evidence of the spread of infection among insects in the field. During the experiments the weather was hot with frequent afternoon showers.

On the application of the Bureau of Science for information regarding the practical success with *Coccobacillus acridiorum* obtained in other countries, the following reports from consuls in Argentina, Columbia, and Algeria were transmitted through the Government at Washington to the Bureau of Science. These are given verbatim.

DEPARTMENT OF STATE,
Washington, May 13, 1914.

The Honorable the SECRETARY OF WAR.

SIR: Referring to your letter of October 1 and the Department's reply of October 10 last, I have the honor to inclose for your information a copy of a despatch from the American Charge d'Affaires at Buenos Aires, reporting on the experiments made in the Argentine with the Coccobacillus d'Herelle.

I have the honor to be, sir,

Your obedient servant,

For the Secretary of State:

ROBERT LANSING, *Counselor.*

Inclosure: From Argentine Republic, No. 272, April 9, 1914.

LEGATION OF THE UNITED STATES OF AMERICA,
Buenos Aires, April 9, 1914.

No. 272.

The Honorable, the SECRETARY OF STATE,
Washington.

SIR: Referring to the Department's instruction No. 91, of October 10, 1913 (File No. 105 P. I. 14), instructing the Legation to secure further information regarding the use in this country of bacteria cultures for destroying locusts, I have the honor to report that a commission appointed by the Minister of Agriculture has decided that Coccobacillus acridiorum d'Herelle, the bacteria sent by the Legation to the Department at the request of the Philippine Government, did not produce favorable results in this country.

M. d'Herelle has severed his connections with the Argentine Ministry of Agriculture and has gone to Europe. He claims that he was not given a fair opportunity to test the result of his bacteria and that he was hampered in his work by the hostility of the minor officials of the Agriculture Department. It is announced that the Ottoman Government wishes him to test his bacteria in Asia Minor.

I have the honor to be, sir,

Your obedient servant,   GEORGE LORILLARD,
Charge d'Affaires ad interim.

[First indorsement.]

B. I. A., WAR DEPARTMENT,
Washington, D. C., May 18, 1914.

Copy to the Governor-General of the Philippine Islands, Manila, P. I., reference being had to letter from this Bureau of May 9, 1914.

Copy for The Director of the Bureau of Science, June 24, 1914.

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6478–37

WAR DEPARTMENT,
BUREAU OF INSULAR AFFAIRS,
Washington, May 9, 1914.

From: Bureau of Insular Affairs.

To: Governor-General of the Philippine Islands.

Subject: Experiments with d'Herelle bacillus for destruction of locusts.

1. In connection with this bureau's letter to you of May 7, and con-

firming telegram from this office of even date, I quote below letter received from the State Department, dated May 6, 1914:

"Referring to previous correspondence on the subject of the use of the d'Herelle bacillus for the extermination of locusts, I have the honor to say that the Department has received a telegram from the American Minister at Bogota, dated the 2nd instant, in which he states that exhaustive experiments for the extermination of the locust have recently been completed by the Colombian Central Commission, and that they proved that the d'Herelle bacillus was effective but the application of it was not practical.

Mr. Thomson added that he was informed that experiments made in January in the Argentine Republic, under the supervision of d'Herelle himself, gave the same results and that the efforts now being made must look to the discovery of a practical method of disseminating the germ. Mr. Thomson further stated that he would forward reports just published by the Colombian Central Commission."

(Sgd.)   CHAS. C. WALCUTT, Jr.
Assistant to Chief of Bureau.

Copy for The Director of the Bureau of Science, June 24, 1914.

WAR DEPARTMENT, BUREAU OF INSULAR AFFAIRS,
Washington, May 7, 1914.

From: Bureau of Insular Affairs.
To: the Governor-General of the Philippine Islands.
Subject: Experiments with d'Herelle bacillus for destruction of locusts.

1. Reference is had to your telegram of the 7th ultimo and to this bureau's reply of even date, relative to the results obtained in the Argentine Republic, Colombia, and Algeria, by the use of d'Herelle bacillus for destruction of locusts.

2. The Bureau is now in receipt of a communication from the State Department reading as follows:

"Referring to previous correspondence with your Department concerning the inquiry made by the Philippine Government as to the results obtained in the Argentine Republic, Colombia, and Algeria, by the use of d'Herelle bacillus for destruction of locusts, I have the honor to say that the Department received a telegram, in reply to its telegraphic instruction, from the American Charge d'Affaires at Buenos Aires, dated the 25th ultimo, stating that the results obtained in the Argentine had not been satisfactory.

The American Consul at Algiers reported by cable on the 28th ultimo that no experiments had been made on locusts by the use of the d'Herelle bacillus, but that the results, from its use on grasshoppers, were inconclusive.

The Department has so far received no reply to its telegraphic instruction to the American Minister at Bogota."

(Sgd.)   CHAS. C. WALCUTT, Jr.
Assistant to Chief of Bureau.

Copy for The Director of the Bureau of Science, June 12, 1914.

DEPARTMENT OF STATE,
Washington, May 21, 1914.

The Secretary of State presents his compliments to The Honorable the Secretary of War, and has the honor to transmit, for the information of the Philippine Government, a copy of a report received from the American

Consul at Algiers, Algeria, giving the result of experiments that have been made in Algeria with Coccobacillus acridorium of d'Herelle..

Reference is made to a letter on this subject, dated April 18, 1914, from the Assistant Secretary of War.

Inclosure: From Algiers, May 2, 1914, with inclosure.

105 P. I./92.

[First indorsement.]

BUREAU OF INSULAR AFFAIRS,
May 23, 1914.

To the Governor-General of the Philippine Islands, Manila, P. I.

CO.

Incl. 6478–41.

[Second indorsement.]

THE GOVERNMENT OF THE PHILIPPINE ISLANDS,
EXECUTIVE BUREAU.

Lc

MANILA, *June 29, 1914.*

Through the Director of the Bureau of Science, to the Director of Agriculture.

EXPERIMENTS IN ALGERIA ON GRASSHOPPERS WITH THE COCCOBACILLUS ACRIDORIUM OF D'HERELLE

In 1913 Professor Sergent of the Pasteur Institute of Algeria commenced investigations to ascertain whether the destruction of the grasshopper indigenous in Algeria, the Stauronotus maroccanus Thunberg, could be effected by the bacillus of d'Herelle.

As the results obtained were considered inconclusive further experiments are being made at present the results of which will be duly reported when experiments are concluded.

The following information was obtained through a personal interview with Professor Sergent.

It was found that injections of cultures containing the bacillus d'Herelle sometimes caused the death of grasshoppers in from 24 to 36 hours but that in other instances injections were not fatal. It was further found that the virulence of bacillus was increased in the bodies of grasshoppers and that by injections of bacillus from one grasshopper to another the virulence of the bacillus was increased to such a degree that death could invariably be caused within four hours of infection. After transmission of virus through 28 insects the average life of insect after infection was seven hours, after transmission through 70 insects 6 hours, and after transmission through 100 insects four hours.

The process of infection from one grasshopper to another had to be carried on 20 days to obtain a regular mortality in seven hours after infection, one month for six hours and seven weeks for four hours.

Two tests were made by Professor Sergent in the Department of Oran in 1913 to determine the practical value of infection of grasshopper with the bacillus d'Herelle.

From the 15th to the 22d of April an area of about 40 acres was sprayed with 37 liters of bouillon containing virulent bacilli of d'Herelle, in the line of march of a column of grasshoppers. From the first day that spraying was commenced dead grasshoppers were found infected with bacillus. On the 30th of April large numbers of dead grasshoppers were found but the great majority of insects constituting the column were not affected.

A second experiment was made from the 13th to the 21st of May with a more virulent virus which had been transmitted through from 74 to 87 grasshoppers. Ninety-three liters of bouillon were sprayed with the apparatus of vermorel [Vermell?] over an area of about 80 acres. On the 28th of May very large numbers of dead grasshoppers were observed averaging about 5 grasshoppers per square meter of area sprayed along the course of a brook which probably arrested the advance of the column for some time; the bodies of insects were piled up in heaps.

On the other side of the ravine through which the brook flowed a field of wheat was occupied by the column among which grasshoppers were captured whose intestines contained the bacillus d'Herelle but which showed no sign of sickness.

The grasshoppers located in the field of grain were observed during three weeks prior to a further flight which could not be followed. Every morning considerable numbers of dead grasshoppers were found but no noticeable diminution in number of living grasshoppers could be observed.

It would appear that when part of a column of grasshoppers is infected by spraying area over which column passes further infection ensues owing to the dejections of diseased insects. The experiments made tend to show that infection is not spread by contact or by the eating of dead bodies.

It was admitted by Professor Sergent that the results so far obtained with the bacillus d'Herelle were less satisfactory than the results obtained by other methods of destruction currently employed.

It was ascertained from Professor Trabut, the Director of the Botanical Service of Algeria, that the report of Professor Vermell, the Government Professor of Agriculture of the Department of Oran, who observed the field tests was decidedly unfavorable and that he himself considered the tests so far made to be inconclusive.

No experiments have been made in Algeria on locusts with the bacillus d'Herelle as no locusts have been found in the country since investigations were first commenced.

<div align="right">

DEAN B. MASON,
American Consul.

</div>

ALGIERS, ALGERIA, *May 2d, 1914.*

<div align="right">

AMERICAN CONSULATE,
Algiers, Algeria, May 2, 1914.

</div>

Subject: Transmission of report on Experiments in Algeria in destroying grasshoppers with the Coccobacillus acridorium d'Herelle.

The Honorable the SECRETARY OF STATE,
Washington.

SIR: Referring to Department cable of April 28th directing that a brief report be made by cable as to the results obtained with the locust bacillus d'Herrelle and to the reply of this Consulate stating that experiments have not been made on locust and that results with grasshoppers were inconclusive I have the honor to submit herewith a more detailed report as to the experiments that have been made in Algeria with the Coccobacillus acridorium d'Herelle.

I have the honor to be, sir,
Your obedient servant,

<div align="right">

DEAN B. MASON,
American Consul.

</div>

Inclosure: Report in triplicate.

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MALARIA IN THE PHILIPPINE ISLANDS

II. THE DISTRIBUTION OF THE COMMONER ANOPHELINES AND THE DISTRIBUTION OF MALARIA [1]

By M. A. BARBER, ALFONSO RAQUEL, ARISTON GUZMAN, and
ANTONIO P. ROSA

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

TWO PLATES AND 1 TEXT FIGURE

This work logically follows that of Walker and Barber [2] on the transmission of malaria in the Philippine Islands. In this work the infectivity to malarial parasites of *Anopheles* (*Pseudomyzomyia*) *rossii, Anopheles* (*Myzomyia*) *febrifer, Anopheles* (*Myzorhynchus*) *barbirostris, Anopheles* (*Myzorhynchus*) *sinensis,* and *Anopheles* (*Nyssorhynchus*) *maculatus* was compared by feeding experiments on gamete carriers. Some 184 feeding experiments and the dissection of some 1,287 mosquitoes were carried out with special reference to the relative infectivity of different species. It was found that of 162 specimens of *Anopheles febrifer* dissected 108, or 66.66 per cent, were infected; of 3 *A. maculatus*, 2, or 66.66 per cent, were infected; of 187 *A. rossii*, 35, or 18.71 per cent, were infected; of 100 *A. barbirostris*, 6, or 6 per cent were infected; and of 12 *A. sinensis* none were infected. These numbers and percentages are based on certain strictly comparative experiments in which the different species were fed at the same time on the same patient, in which only females that were known to have sucked blood were considered, and in which it was known that the patient's blood con-

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[1] Read before the Philippine Islands Medical Association, November 6, 1914.

[2] *This Journal, Sec. B* (1914), **9**, 381–439.

tained viable gametes at the time of experiment from the fact that at least one mosquito at each feeding became infected. Taking into consideration all of the experiments, the numbers and percentages are as follows:

Species.	Dissected.	Infected.
		Per ct.
Anopheles febrifer	373	35+
Anopheles maculatus	49	6+
Anopheles rossii	642	10—
Anopheles barbirostris	205	3+
Anopheles sinensis	18	0

With regard to sporozoites in the salivary glands the numbers are as follows:

Species.		Salivary glands.	
	Dissected.	Infected.	
		Number.	Per cent.
Anopheles febrifer	111	20	18+
Anopheles maculatus	1	1	100
Anopheles rossii	125	0	0
Anopheles barbirostris	75	0	0

Taking into consideration these percentages and the numbers and distribution of anophelines as far as then known, it was concluded that *Anopheles febrifer* was probably the chief carrier, or one of the chief carriers, in the Philippine Islands.

The object of the present work has been to study more widely the distribution of the commoner anophelines of the Archipelago and the distribution of endemic malaria as determined by the parasite and spleen indexes of children, for the most part of children ten years and less of age. Special attention was given to the relation between the breeding places of anophelines and the presence of malaria. The contiguity of the breeding places of anophelines to inhabited places, the character of surroundings, and other factors were also studied with the aim of determining why one locality is malarious and another not. Data were obtained on the habits of anopheline larvæ and imagoes, and some experiments in the destruction of the larvæ of *Anopheles* in running streams were carried out. In short, our aim has been to establish a scientific basis for combating malaria in the Philippine Islands.

THE DISTRIBUTION OF ANOPHELINES

LARVÆ

In determining the number and character of the larvæ of a locality, the following method for the most part was employed: Larvæ were collected in jars and bred, and the species were determined from the imagoes. In a large proportion of the localities for which the malarial index was determined, the character of the mosquito fauna and the breeding places of the mosquitoes were also surveyed. An anopheles-mosquito survey was made of many other localities. For the determination of some species we are under obligations to Prof. C. S. Banks, entomologist of the College of Agriculture of the Philippine Islands. In general, we have found the relative numbers and habitats in various provinces much the same as they are in the neighborhood of Canlubang, Laguna Province, Luzon, which was very thoroughly surveyed by Walker and Barber.[3] *Anopheles rossii* is by far the most abundant and the most widely spread. *Anopheles febrifer* and *A. barbirostris* come next in order of abundance; of these, *A. febrifer*, although of more restricted habitat, probably exceeds *A. barbirostris* in abundance, because of the far greater numbers of larvæ found in the breeding places. *Anopheles sinensis* probably ranks fourth. Its relative abundance is rather difficult to determine, since it is preferably a rice-paddy breeder, and may occur in considerable numbers under certain circumstances. *Anopheles maculatus* is probably the least plentiful of the five species, since its breeding places are restricted, and relatively few mosquitoes are found in them. A wider search conducted in each locality through all months of the year may change the order given for the last two, but probably not for the first three. Thus far no other species has been found in any considerable abundance.[4]

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[3] Loc. cit.

[4] Specimens of anopheles from larvæ collected in a brook at Buhisan, Cebu, were sent to Dr. C. S. Ludlow, of the Army Medical Museum, Washington, D. C., who identified them as *Myzomyia parangensis* Ludlow. These larvæ were very plentiful in the brook at Buhisan, and have been found by one of us (Barber) in Bugsanga River, Mindoro, where they occurred in abundance in May. Specimens from this lot of mosquitoes were identified by Mr. C. S. Banks, entomologist of the College of Agriculture of the Philippine Islands, as *Myzomyia rossii*. In view of the rather indefinite status of this species, we have in this paper taken the more conservative ground and included it and some very similar forms under the name *Anopheles (Myzomyia) rossii*. We believe that breeding experiments with this and similar doubtful forms should be carried out in order to determine

Details as to the distribution of the various species in different localities will be found in Table I, but in general, *Anopheles rossii* and *A. febrifer* have been found in nearly all localities where careful search has been made for them. *Anopheles febrifer* has been found in various parts of Luzon, Mindoro, Palawan, Cebu, and Negros Islands—practically in all localities where fresh flowing water has occurred in ditches, brooks, and rivers. *Anopheles maculatus* has been found in Luzon (Canlubang, Camp Stotsenberg, Antipolo, and near Taytay), Mindoro (San Jose), and Cebu (near the city of Cebu). *Anopheles sinensis* has thus far been found only in Laguna Province, Luzon; but it is probable that a search throughout rice paddies at different seasons would prove that it is widely distributed in the Archipelago.

As to habitat *Anopheles rossii* is by far the most adaptive. It has been found in the very salt water of evaporating ponds used in obtaining salt (Parañaque) and in brooks flowing from springs. It occurs in temporary puddles at roadsides and in the largest rivers. It is the commonest anopheline of rice paddies. While preferring moderately fresh water, it is often found abundantly in foul pools, carabao wallows, and even in the very foul water of tanks containing soaking cane (Canlubang). It has been found in small pools among stones practically in the middle of a clear flowing brook, where no algæ or other vegetation occurred except the small amount growing on the stones (Cebu). In general, it is a sun-loving species and is rarely missing where masses of algæ in ponds or rivers are well exposed to the sun.

Anopheles barbirostris, while very widely distributed, has a more restricted distribution than *A. rossii*. It is less often found in foul water and is commonest where the water is comparatively fresh and aquatic plants are abundant. We have found it in a pool of brackish water separated from the sea by a railroad embankment (Tayabas), in a succession of pools of different grades of saltiness in the bed of a nearly dry stream, and in

the amount of variation possible in the offspring of a single pair of mosquitoes. It may well be that only by such experiments will a sound basis for the classification of some species of anopheles be obtained. As stated by Walker and Barber [*This Journal, Sec. B* (1914), 9, 439, note] specimens of *Anopheles* (*Myzomyia*) *febrifer* Banks were sent by us to Doctor Ludlow, who identified them as *Myzomyia christophersi* Theobald. Doctor Ludlow informed us by letter that this is the same species as that reported by her from the Philippines as *M. funcsta*. [See Ludlow, Bulletin No. 4, War Department. Office of the Surgeon General (1913), p. 36, footnote.] *Myzomyia christophersi* is a well-known malaria carrier of the hill regions of India.

the apparently pure salt water of the inflowing tide at the mouth of a river (Tayabas). It is very often associated with *A. rossii* in masses of algæ exposed to the sun, especially in rivers.

Anopheles febrifer is more restricted in habitat than either of the foregoing species. We have never found it in brackish water. It shows a decided preference for clear flowing water, especially where there is an abundance of overhanging grass, roots, or other vegetation at the margin. It is rarely found except at the banks of the stream or at the edges of islets of grass or the like, and when liberated in the center of the stream the larvæ usually wriggle rapidly to the shaded margin. It prefers brooks and small streams to large rivers, although it may sometimes be found at the margins of the latter, especially if a steep bank or grass or other vegetation offers shelter. It often occurs in small indentations in the shore or along the banks of slightly widened parts of a stream where quieter water is found. It almost never occurs where a current strikes the banks; small dams, often made in small streams to obtain a pool for laundry purposes, afford a favorite shelter. Although preferring vegetation at the margin of a stream, *Anopheles febrifer* may sometimes be found on a bare clay bank, especially if it is slightly overhanging. We have found it in small numbers among stones at the margin of a river (Palawan) and on stones at the walled sides of ditches in the streets of a town (Lilio).

This species tends to avoid the presence of decaying vegetation in water beyond a certain degree. It is usually absent in small bayous of half a meter to a few meters in length, extending from brooks in which it is abundant. Where a brook broadens to a width of a meter or more, offering quiet water at the margins, *A. febrifer* is often found abundantly, but if the stream widens to a broad pond, the larvæ become rare or absent. Where the same brook becomes swiftly flowing again, the larvæ reappear. We have not found them in pools or ponds without outlet. A certain amount of sewage or other animal matter in water is tolerated. We have found the species abundantly some rods below the exit of a large septic tank (Canlubang), but not immediately below. It also occurs, often in considerable numbers, in ditches serving as open sewers, these containing clear water with a small amount of sewage. We have found it in a brook into which water was seeping from pits containing soaking hides (Magdalena). Although usually found in clear water, it may occur in brooks rendered turbid by carabaos (Cebu). *Anopheles febrifer* has not been found in rice paddies, and the flow of water over extensive rice paddies seems to unfit it for this species—a very important

matter in the epidemiology of malaria in regions where rice lands are centers of population.

While brooks or small rivers are preferred as breeding places, *Anopheles febrifer* may occur in streams of very small flow. In one stream there was at that season no flow above ground, but the larvæ were found near the exit of a spring (Mindoro). They have been found in swamps, but only where there was a clear stream flowing over the swampy surface.

While preferring shaded places, the larvæ are often found in brooks and ditches exposed to the sun, but only where there is some vegetation or other protection at the margin.

The general character of the soil does not seem materially to affect the breeding of *Anopheles febrifer*. It has been found in brooks in the red soil of Bataan Province, in sandy streams of Pampanga, in streams worn in volcanic rock in Laguna, in the limestone region of Cebu, and in the stiff clay region of Mindoro. It has been found abundantly at considerable distances from any human habitation, and in ditches flowing between and under houses in large towns.

The two localities in which *Anopheles febrifer* has been found most abundantly are two small rivers, one in southern Mindoro and one in Negros. In the latter locality, a clear brook with steep banks and much vegetation, sometimes between 100 and 200 larvæ could be taken up at one dip of a small collecting pan.

The number of larvæ in a given part of a stream may vary from time to time, apparently independently of any change in conditions. This we have found to be the case in some streams at Canlubang, which we visited many times in the course of about five months for the purpose of collecting mosquitoes for feeding experiments. We found wide variations in the frequency of other species also.

This species is sometimes found in association with *Anopheles barbirostris* in streams with abundant vegetation. It may often be found at the margin of a stream, while *A. rossii* is plentiful in algæ at the center. We have sometimes found it in nearly "pure culture" at the steep shaded margin of a large brook, while *A. barbirostris* and *A. rossii* occurred at the more exposed margin at the other side. In a certain brook (Negros) *A. febrifer* was abundant at the margin, while *A. rossii* alone occurred in carabao tracks containing water and exposed to the sun only a few centimeters from the margin of the brook.

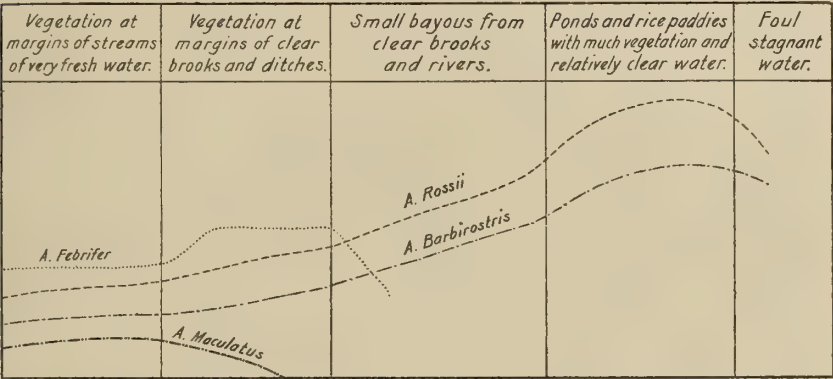Fig. 1 shows the relative frequency of the larvæ of four species of *Anopheles* in habitats arranged according to the amount of decaying organic matter in solution in the water.

Of course, the distribution as given in fig. 1 is true only in a very general way and takes into account only one factor: namely, the amount of decaying vegetable matter in the water. For example, seepage from small springs, or the overflow of wells, often fills animal tracks or small depressions in the soil with comparatively fresh water. Where exposed to the sun, these pools often contain many larvæ of *A. rossii* and of no other anopheles. It must be borne in mind, also, that where a species is very abundant it may overstep the limits commonly fixed where the numbers are only normal. Furthermore, in times of scarcity of water eggs may be laid in less preferred places. However, in both the dry and wet seasons we have found the distribution of *A. febrifer* comparatively limited, and we have not found it except where the water was kept comparatively fresh by a

Fig. 1. The relative abundance of four species of *Anopheles* in habitats arranged according to the amount of decaying organic matter in solution in the water.

spring or some flowing stream. We have frequently found larvæ of mosquitoes other than anopheles in water contained in the axils of leaves, in upright joints of bamboo, or in coconut shells, but never anopheles, although such occurrence of anopheles has been reported by others in the Philippines.

Anopheles maculatus has been found in about the same sort of habitat as *A. febrifer*, but is far less common and apparently much more restricted to very fresh water. It has been found in localities at elevations of from 100 to 200 meters, such as Antipolo and a brook near Taytay, Rizal Province; Camp Stotsenberg, Pampanga Province; and Buhisan, Cebu Province; but it occurs near sea level at San Jose, Mindoro, and not far above sea level at Canlubang, Laguna Province. In Mindoro it was found among aquatic plants in a large irrigation ditch exposed to the sun. The larvæ from these patches of weeds were care-

fully kept separate and bred in a separate jar, and proved to be *A. maculatus.*

Anopheles sinensis, while not so carefully studied as the preceding species, appears to occur in about the same sort of localities as *A. barbirostris,* but is much less frequently found.

We have found larvæ of *A. febrifer* and *A. barbirostris* breeding in mountain brooks the temperature of which was 23° C. and in brooks with a temperature of 28° C. *Anopheles rossii* is often found in small shallow pools exposed directly to the tropical sun where the temperature is much higher.

The different seasons in the Philippines affect the breeding of anopheles chiefly through diminution of breeding places in the dry season and the flushing of streams during the wet season. We have found very young larvæ of anopheles, apparently of *A. febrifer,* during freshets, in eddies among floating débris. Apparently enough eggs or larvæ remain in a stream after a freshet to restock it, even where no females are at hand to deposit new eggs. *Anopheles rossii* may be found in roadside puddles or ditches during the rainy season. The stream breeders, *A. febrifer* and *A. maculatus,* require more permanent conditions, and their breeding places are consequently more restricted. We have found *A. febrifer* in considerable quantities during every month of the year. The year 1914 was somewhat exceptional in the Philippines in the lack of long-continued rains during the wet season, and this condition favored the stream breeders, since they were less disturbed by freshets. In the artificial ditches of running water, with which some towns are abundantly supplied, the amount of flow varies less from season to season than in brooks, so that the breeding of anopheles is less interfered with there.

IMAGOES

A matter of much importance in the dissemination of malaria is the behavior of mature anopheles, especially with reference to their habits of visiting houses and biting human beings. The avidity for human blood of anopheles in captivity is shown by Walker and Barber,[5] who give the following percentages for females that took advantage of one opportunity to suck blood:

	Per cent.
Anopheles rossii	63.6
Anopheles barbirostris	49.4
Anopheles febrifer	54.8
Anopheles maculatus	50.0
Anopheles sinensis	67.8

[5] Loc. cit.

With regard to the avidity for human blood and the habit of house visiting of anopheles under natural conditions, we have data from two localities: namely, the San Jose Estate, Mindoro, and the Iwahig penal colony, Palawan.

The region occupied by the San Jose Sugar Estate is comparatively flat and not much above sea level. It was formerly covered with cogon grass. Some three years ago it was noted for a very large amount of malaria with a high mortality, but at the present time very severe cases are few and the mortality from malaria is almost nil. Latent malaria is still present to a considerable degree, as shown by the figures in Table I. Barber spent the month of May, 1914, on the estate, and during this time a squad of assistants made visits almost daily to houses belonging to the estate for the purpose of catching mosquitoes, especially anopheles. The mosquitoes were caught in test tubes or by means of nets of gauze or cobweb and were brought fresh, often living, into the laboratory for identification. Dr. G. W. Daywalt, resident physician of the estate, has for many months kept mosquito catchers employed on the estate, and he ascribes much of the diminution of malaria to this part of the work. He kindly put at our disposal his squad of trained mosquito catchers to assist our own employees. We take this opportunity of acknowledging this and other courtesies shown us by Doctor Daywalt during our visit to the estate.

The results of the mosquito catching are given by barrios in Table I. The number of days on which mosquitoes were caught, 25 in all, are given for each barrio. When morning and afternoon visits were made on the same day to a given locality the two visits are recorded as one.

It is seen that the ratio of *Anopheles febrifer* to *A. rossii* varies greatly in different barrios. Two barrios, "J" and Magbando, show a relatively large number of *A. febrifer*. Barrio "J" was at that time a small barrio of only seven or eight houses. It is comparatively new and is situated about 2 kilometers from the center of population of the estate. There was a minor outbreak of malaria in this barrio earlier in the season, and the general locality has in the past been notably malarious. Larvæ of *A. febrifer* were found by us in moderate numbers in a ditch fed by the seepage from an irrigation canal near this barrio, and they also occurred in small numbers in grass at the edge of the very swift water of the canal itself. They were abundant in a brook flowing through a wooded swamp less than half a kilometer distant. Some oiling of the seepage ditch near the barrio had been done, but little or none was done farther up this ditch or

in the swamp. The inhabitants are all Filipino laborers and their families.

Magbando barrio consists of only two or three houses at the edge of or just outside of the estate. A clear stream containing numerous larvæ of *A. febrifer* flows through the woods a few meters from the houses. Little oiling has been done in this region, since it is far from the center of population of the estate. The small population consists of Filipinos and Filipino-Mangyan mestizos.

In the other barrios *Anopheles rossii* considerably outnumbers *A. febrifer*. Bugsanga barrio includes a long string of from 15 to 20 buildings including some small dormitories and is situated on the bank of a large river, the Bugsanga. Numerous larvæ of *A. rossii* and comparatively few of *A. febrifer* were found by us in this river. There is also an irrigation ditch entering this river in the upper part of the barrio. The population of the barrio is relatively small and includes both Americans and Filipinos. This barrio is at the edge of the cultivated part of the estate and is about 1 kilometer from the center of population, so comparatively little oiling has been done in the river.

The barrios of Mindoro and Lubang include the largest part of the population of the estate. They are situated on the banks of a small winding stream, Magbando River, which in places has swampy banks. There are numerous irrigation ditches in the neighborhood and small streams formed by seepage from these ditches. We found larvæ of *A. febrifer* in small numbers at the edge of this stream and some of *A. rossii* in the swampy ground. The various streams and ditches have been faithfully oiled, and the larvæ are remarkably few considering the difficulty of destroying them over such a large and abundantly watered area. Mindoro barrio has about 140 houses. Lubang has about 75 houses including some small dormitories. The population of both barrios is largely Filipino with a small percentage of Japanese and a few Americans.

Dormitory H, situated at one end of Lubang barrio, is here considered separately, since the conditions were somewhat different from those of the other houses of the barrio. The building, except the kitchen, is screened. It is comparatively well shaded and is near Magbando River on one side and a seepage ditch on the other, neither of which showed many larvæ. A considerable percentage of the anopheles caught in this building were found in the screened vestibule. On some nights this vestibule was purposely made into a mosquito trap by leaving the

outer door open and keeping the inner door carefully closed. Twenty-two specimens of *Anopheles rossii* were caught in this vestibule at one time. It is worthy of note that nearly all of the mosquitoes caught in this vestibule were females containing blood, so they must have obtained their blood elsewhere and have been later attracted by the lights or people in the dormitory.

Fewer visits were made to some barrios than to others, and the number of houses visited varies, so that Table I shows only approximately the frequency of anopheles in each locality, but the data for the proportion of the several species are more nearly accurate. No reliable data as to the length of flight of anopheles could be obtained in this region, since there was no barrio which did not have a possible breeding place within a few meters. The ground is comparatively open with few trees or underbrush to shelter mosquitoes or to retard their flight.

In Table I are given data showing the amount of latent malaria found in these barrios. The presence of malaria was determined by the percentage of parasites found in blood smears. No intentional selection was made of the sick in obtaining samples. People of all ages were examined as they could be obtained. An examination of all persons in each barrio could not well be made, and the inhabitants of the different barrios, with the exception of Magbando, tend to shift more or less from one locality to another, so no very definite correlation between the mosquito fauna and the amount of indigenous malaria in the several barrios could be expected. Of the two barrios showing a relatively large proportion of *Anopheles febrifer*, barrios "J" and Magbando do not show a larger percentage of indigenous malaria than the other localities. However, Magbando contains a population that has long resided in a malarious region and may have acquired some immunity. Among the nine negatives, the blood of three showed indications of anæmia, possibly resulting from previous attacks of malaria. Barrio "J" is a comparatively new barrio, and as stated above exhibited a minor epidemic of malaria earlier in the season. The percentage of positive cases, however, was only 33.3, which is slightly below the average of the whole population. Moreover two examinations at different dates were made of a part of the people of this barrio and all positives included in the percentage. The lower part of Mindoro barrio has many more breeding places than the upper, and whether by coincidence or not, the lower streets showed a much greater amount of latent malaria than the upper.

The proportions of sexes of the different species of anopheles and the proportion of females containing blood in the stomach

are shown in Table II. All anopheles caught are included in this table, so that the total somewhat exceeds that of Table I, which includes only those of which the place of origin was known. The great preponderance of females and the large proportion of these which had taken blood are so apparent in the table that no further analysis is necessary.

TABLE I.—*Anopheles and indigenous malaria in the various barrios of San Jose Estate, Mindoro. Mosquitoes were caught in houses during May, 1914.*

Barrio.	Houses (approximate).	Days on which visits were made.	Mosquitoes caught.					Persons examined for malarial parasites.	
			A. febrifer.	A. rossii.	A. barbirostris.	A. maculatus.	Total.	Number.	Positive.
									Per ct.
"J"	6–8	11	166	34	0	0	200	48	33.3
Magbando	2–3	4	34	14	0	0	48	11	18.2
Bugsanga	15–20	10	20	110	0	1	131	16	56.3
Mindoro	125–150	15	13	122	0	0	135	127	37.0
Lubang	70–75	12	17	69	1	0	87	15	46.7
Dormitory H with kitchen		17	1	192	0	0	193		
Total			251	541	1	1	794	217	37.3

TABLE II.—*Anopheles caught in houses during May, 1914, in San Jose Estate, Mindoro.*

Species.	Females.			Males.	Total.	Females containing blood.
	With blood.	Empty.	Doubtful.			
						Per ct.
Anopheles febrifer	232	3	13	1	249	93.5
Anopheles rossii	452	30	37	53	572	87.1
Anopheles barbirostris	1	0	0	0	1	100.0
Anopheles maculatus	1	0	0	0	1	100.0
Total	686	33	50	54	a 823	89.2

a Total exceeds that of Table I, because some are included here the distribution of which was doubtful.

The Iwahig penal colony, Palawan, was visited by Barber in June, 1914. This colony occupies a considerable territory, much of it comparatively flat and elevated from a few centimeters to 7 meters above high tide. Like the San Jose Estate, most of the colony land has been cleared of jungle within a few years, and some parts of it are still being cleared. The whole region has long been known as very malarious.

Owing to the proximity of a high range of mountains the region is well watered. A large river with numerous bayous flows through the colony grounds, and the region is well supplied with brooks of clear water.

A large part of the land is devoted to coconut trees, but there are some irrigated rice fields. Larvæ of *Anopheles rossii,* *A. febrifer,* and *A. barbirostris* were found in the river and in various brooks in the colony.

The time at our disposal at Iwahig was too short to organize an effective corps of mosquito catchers, so all imagoes caught were obtained inside of sleeping nets. These nets, where used by the prisoners, were often badly adjusted, and mosquitoes could enter under the edges or from beneath where the sleeping mats failed to cover the floor or the bottoms of the bunks. The record of mosquitoes caught is given in Table III. Here both the anopheles of different species and, under the head of "*Culex,*" mosquitoes not anopheles are included. Dates of catches are given, different buildings are noted, and in one column only the mosquitoes caught in a certain bed are included. On certain mornings the bed nets were left down so as to facilitate the capture of mosquitoes. Each bed was occupied by only one person.

The houses on the river bank are from 5 to 7 meters above the large river noted above in which larvæ of *A. febrifer, A. rossii,* and *A. barbirostris* were found. The brigade dormitory is a large building separated from the houses on the river bank by a nearly open parade ground approximately 160 paces broad. A careful search, continued through several days, showed that there was at that time no mosquito breeding place nearer the brigade dormitory than the river. So the distance of flight to this dormitory must have been at least 170 paces. In accounting for the fewness of mosquitoes in the brigade dormitory, there must be taken into account not only the distance of flight, but also the fact that mosquitoes in coming from the river are naturally first attracted to the houses along the river bank. Again, the brigade dormitory is partially inclosed, and the beds occupy three stories. On the third floor of this building 39 nets at one collection yielded only 7 specimens of culex and none of anopheles. The beds in the houses on the river bank, on the other hand, were on the veranda, a little above the ground level.

Balsahan and Esperanza are small barrios of the colony. The beds are in small dormitories but little above the ground level and only a few rods from brooks where larvæ of *A. febrifer* were found.

TABLE III.—*Mosquitoes caught in bed nets at Iwahig penal colony, Palawan.*

Date.	Houses on river bank.		House L.	Brigade dormitory.[b]	Balsahan and Esperanza.
	Veranda, house H.				
	Bed No. 1.	Total.			
June 18, 1914	*A. febrifer:*[a] ♀ with blood — 9; ♂ — 0 / *Culex:* ♀ with blood — 2; ♂ — 0	*A. febrifer:*[a] ♀ with blood — 17 / *Culex* — 22		*Anopheles* — 0 / *Culex:* ♀ — 10; ♂ — 0	
June 19, 1914	*A. febrifer:*[a] ♀ with blood — 18 / *Culex* — 0	*A. febrifer:*[a] ♀ with blood — 32 / *Culex* — 7			*A. febrifer:*[a] ♀ with blood — 8; ♂ — 0 / *Culex* — 1
June 20, 1914	*A. febrifer:*[a] ♀ with blood — 20; ♂ — 1 / *Culex:* ♀ with blood — 13; ♂ — 1		*A. febrifer:*[a] ♀ with blood — 6; ♂ — 0 / *Culex* — 1	*A. febrifer:* ♀ with blood — 4 / *A. barbirostris:* ♀ with blood — 3 / *A. rossii:* ♀ with blood — 3 / *Culex:* ♀ mostly with blood — 165; ♂ — 57	
Total	*A. febrifer* — 27 / *Culex* — 2	*A. febrifer* — 70 / *Culex* — 43	*A. febrifer* — 6 / *Culex* — 1	All *Anopheles* — 10 / *Culex* — 232	*A. febrifer* — 8 / *Culex* — 1

[a] No other species of *Anopheles* found.

[b] About 175 beds.

In proportion to the population a larger number of acute cases of malaria in Iwahig come from the outlying barrios than from the central or better improved part of the colony. This is probably due in part to the more exposed situation of the newer barrios, and possibly in part to the fact that the quinine prophylaxis practiced in the colony (5 grains per day to each person) could not be so rigorously carried out in the barrios. In order to give some idea of the amount and origin of acute cases of malaria, a record is given in Table IV of the admission of fever cases to the colony hospital during three days. The microscopical examinations were made by Barber.

TABLE IV.—*Cases of acute malaria admitted to Iwahig hospital during three days of June, 1914.*

Date.	Pa-tient.	Resi-dence in the colony.	Residence when taken ill.	Microscopical findings in blood.
1914.		*Yrs. mos.*		
June 22__	7303	9	Malamig barrio_____	Tertian +.
Do___	5627	7	_____do _____	Tertian, rare.
Do___	8123	5 0	_____do _____	Anæmia, parasites doubtful.
Do___	9080	4	Central_____	Negative.
Do___	8184	1	_____do _____	Æstivo-autumnal ++.
Do___	7734	4	_____do _____	Tertian, few.
Do___	2976	5 0	_____do _____	Æstivo-autumnal +.
Do___	6851	1	_____do _____	Æstivo-autumnal, few.
Do___	8845	1	_____do _____	Do.
June 23__	8398	1	Malamig barrio_____	Æstivo-autumnal, rare.
Do___	8811	1	Kabaloan barrio_____	Tertian +.
Do___	7077	1	Esperanza barrio_____	Tertian, rare.
Do___	8701	2	Malamig barrio_____	Æstivo-autumnal, rare.
Do___	5455	1	Esperanza barrio_____	Negative.
June 24__	8597	1	Balsahan barrio _____	Æstivo-autumnal +.
Do___	8657	4	Malamig barrio_____	Tertian +.
Do___	8611	6	_____do _____	Æstivo-autumnal with crescents, rare.
Do___	8784	1	Esperanza barrio_____	Æstivo-autumnal rings, rare, crescents +.
Do___	9016	2	_____do _____	Do.
Do___	8150	3	Quinina barrio _____	Æstivo-autumnal, rare.
Do___	5906	3 0	_____do _____	Negative hæmoglobinuria.
Do___	6840	6	Central_____	Æstivo-autumnal, rare.
Do___	6184	5 0	_____do _____	Æstivo-autumnal +.
Do___	8891	4	_____do _____	Tertian, rare.
Do___	8567	1	_____do _____	Æstivo-autumnal +.

SUMMARY OF TABLE IV.

Examined	25
Parasites found	21 = 84 per cent.
Cases from barrios	15 = 60 per cent.
Cases from Central	10 = 40 per cent.
Average admissions per day	8.3

According to the resident physician, Dr. José Marfori, the admissions during this period somewhat exceeded the average. It is impossible to state what portion of these 25 cases are recurrences. But all prisoners had spent at least two years in Bilibid Prison at Manila before coming to Iwahig. We have received the following information from the Bureau of Health:

Malaria is infrequent among prisoners who have been in Bilibid for two years or more. Occasionally malaria develops, apparently from latent infection, after operation. During the month of December, 9 tubercular patients were returned from Iwahig, among whom 4 were found with the enlarged spleens and blood positive for malarial organisms.

So it is likely that malaria in a large proportion of the cases mentioned in Table IV was contracted in Iwahig.

With regard to the amount of latent malaria at Iwahig we have data of 62 men of the central brigade dormitory, the occupants of which are probably somewhat less exposed to infection than the average population. Only men apparently healthy were examined. Of the 62, 8, or 12.9 per cent, harbored malaria parasites. Of the 8 positives the length of residence in the colony was as follows: Three months, 1 person; eight months, 1; nine months, 1; eleven months, 2; one year, 1; three years, 2.

A noteworthy deduction from the data obtained at Iwahig is the large proportion of *A. febrifer* as compared with other species of anopheles, and the large number of *A. febrifer* found in houses in proportion to the number of larvæ found in the neighboring breeding places. Further, it seems to be demonstrated that the flight of this species is at least 170 paces, although comparatively few got so far.

The data from both Iwahig and San Jose, Mindoro, demonstrate that *Anopheles febrifer*, like *A. rossii*, is a house-seeking species and readily takes blood from human beings. In both San Jose and Iwahig *A. barbirostris* was found in dwellings, and the fact that specimens of *A. barbirostris* containing blood were found in sleeping nets at Iwahig proves that this species will enter houses and bite human beings. In India *A. barbirostris* has been generally considered a "wild" species, little disposed to visit houses.[6] When we compare the small number of this species found in nets in Iwahig with the number of larvæ found breeding in the vicinity, it seems probable that in the Philippines, also, this species has a less tendency to visit houses than either *A. febrifer* or *A. rossii*. Only one specimen of *A. maculatus* was found in

[6] Stephens and Christophers, The practical study of malaria, etc. The University Press of Liverpool (1908), 169, 181.

houses (Bugsanga barrio, San Jose), but the larvæ of this species were so few in that locality that no trustworthy deductions can be made.

Some definite data regarding the lurking places of anopheles, other than houses, were obtained at San Jose, Mindoro, by a study of *A. febrifer*. A clear brook flowing through woods near Magbando barrio contained numerous larvæ of *A. febrifer*, but comparatively few of any other species of mosquito. A search was made for hiding imagoes in the numerous crab holes and in the matted tree roots along this brook. Smoke was blown through a tube into these holes and roots, and the mosquitoes, on being driven out, were caught in a large glass funnel having the neck plugged with cotton. The bottom of the funnel was quickly covered with a large piece of pasteboard, and the mosquitoes, stupefied by means of tobacco smoke, were blown into the neck of the funnel. They could then readily be removed to vials. Numerous specimens of culex were found in these lurking places, but only 2 of *A. febrifer*, although the larvæ of the former were few and of the latter many. Both specimens of *A. febrifer* were females. One was caught in a crab hole but half a meter from a much frequented spring. The other, caught in matted roots, contained about 70 nearly mature ova. No malarial parasites were found in either on dissection. Magbando barrio (see Table I), where 34 specimens of *A. febrifer* were caught in houses, is located only a few rods from the bank where the 2 specimens of *A. febrifer* were caught in the crab hole and in roots.

A careful search for mosquito imagoes was made in Bioos, Negros Island, along the banks of a jungly brook, which contained very numerous larvæ of *A. febrifer*. Banks deeply covered by overhanging vegetation, crab holes, hollow logs, and vegetation of various sorts were "smoked," but very few mosquitoes of any kind were found and none that could be identified as anopheles. Other species of anopheles, *A. rossii* and *A. barbirostris*, were breeding in this brook. Dwellings and the shelters of sheep, horses, and pigs located near the brook were searched at daylight and at various times during the day with negative results. No signs were observed of anopheles flying at daylight to or from the brook or buildings. At that time only a moderate amount of malaria existed among the people living on the coconut plantation bordering this brook, but it is said to have been much more prevalent there before the jungle was cleared away.

Enemies of mosquitoes—myriads of ants and many spider webs—were abundant along the banks of this brook. However, in view of the multitudinous lurking places which a jungle offers,

it is very easy to overlook mosquitoes when they are concealed in the daytime, and it is possible that a large proportion of the stronger larvæ emerged and reached safety.

Similar negative results were obtained in a comparatively short search for mature anopheles in Magdalena, Laguna Province, where *A. febrifer* larvæ were plentiful and malaria very prevalent.

It is possible that certain meteorological conditions favor the dispersal of this species of anopheles as well as that of other species. In Mindoro, where many anopheles imagoes were found, the weather was hot with frequent afternoon showers. At Iwahig, where many mature *A. febrifer* were caught, the temperature was comparatively low for the tropics and the humidity great. At Negros, where negative results were obtained, the weather was hot with occasional showers, not unlike that of Mindoro at the time of our visit there. At Magdalena it was hot and dry. However, the facilities for obtaining mature anopheles were much better at Mindoro and Iwahig than at the other localities examined, and it is probable that a search continued over some time and during a variety of meteorological conditions would reveal the lurking places of any anopheles in a locality where their larvæ are found in any number.

In the Philippines, as well as in other places where observations have been made, anopheles usually remain hidden during the daytime. During many days of work in shady woods and cañons we have observed anopheles flying by day on only one occasion, when a specimen of *A. barbirostris* settled on the hand at noontime on a cloudy day in a narrow cañon. The mosquito filled its stomach with blood and expelled a drop from the anus. The specimen was caught and taken to the laboratory for dissection and identification.

Owing to a lack of time a comparatively small proportion of the mature anopheles caught at Mindoro and Iwahig were dissected and examined for malarial parasites. Moreover the relative infectivity for malarial parasites had already been determined for all the species obtained in these places. All dissections that were made were negative. In Mindoro, where mosquitoes were caught in houses almost daily, the chances of finding an infected one were probably small. In some Panama barracks, where a daily routine of catching anopheles was practiced, Darling[1] reports that only one naturally infected mosquito was found in about 500 dissected.

[1] *Proc. Canal Zone Med. Assoc.* (April, 1909, to March, 1910).

DISTRIBUTION OF MALARIA IN VARIOUS PROVINCES, TOWNS, AND BARRIOS

In determining the malarial index of a locality, both the parasite and the spleen indexes were taken, in a large proportion of cases by the examination of school children, the majority of whom were from 5 to 10 years of age. No intentional selection of well or sick children was made. The examinations were made during school hours of children in attendance on that day. The examination of school children entails two disadvantages. First, the children who are seriously ill are less likely to be in attendance, so there was some selection of those less likely to be malarious. However, as numerous cases showed, children often return to school very soon after their illness, and since comparatively little systematic quinine treatment is practiced in Filipino towns, children recently recovered from malaria are likely to show parasites. Therefore the error through selection of the well is not great. Secondly, the parasite rate is usually higher in children from 1 to 5 years of age than in older children (see Magdalena, Table VI), and since few children below 5 years of age attend school, our percentage would be somewhat lower than for all young children. Neither source of error, however, affects the value of our statistics in the comparison of different localities. Moreover it is improbable that any considerable amount of indigenous malaria can exist in a locality without some cases appearing among children from 6 to 10 years of age. Since our aim was primarily to ascertain the distribution of malaria, the advantage of quickly obtaining specimens from a large number of children in schools would outweigh the disadvantages of this method.

In some cases examinations were made of children, taken in part at least, out of school or of older persons. These cases are indicated in the tables.

Blood for examination for parasites was taken from the ear, and as a rule, but one slide was taken of each person at a visit. A thick smear and a thin smear were made on the same slide. The blood was dried in the air, then the slide was carefully wrapped in a piece of paper on which were recorded the data of the case: namely, name of person, age, sex, result of spleen examination, and history of recent illness. The wrapped slides were closely fitted into pasteboard boxes, and the boxes were inclosed in paper and sent as quickly as possible to the laboratory. These precautions were taken in order to minimize dangers from moisture or overdrying, according to the season,

and to protect the preparations against cockroaches or ants, which will eat the blood on exposed slides. As a rule, the slides reached the laboratory in time to be stained within three days after collection.

At the laboratory the slides were labeled and stained, the data on the slips and the results of the examination were entered in a book, so that it was possible to compare the results obtained from a given individual with those made at a later examination. The names of children positive for malarial parasites were reported to the principal of schools in order that treatment might be given these cases. In staining, the thin portion of the slide was fixed in methyl alcohol and dried. Then, with no fixing or other treatment of the thick smear, the slide was immersed for about one hour in staining jars containing Giemsa-Romanowski stain 1 part to 40 parts of water. The stained preparations were rinsed in distilled water, dried, and examined under the oil immersion without a cover glass.

In determining the presence of parasites, we depended largely on the thick film, although the thin was used where comparison or confirmatory evidence was needed. The examinations were nearly all made by Barber and Guzman, both of whom had had a long previous training in examining thin smears for malaria in the tropics and in comparing the appearance of parasites in the thick smears with that in the thin smears.

In most of our preparations the hæmoglobin was well laked out by the watery stain and the parasites stood out clearly against the bluish or purplish background between the leucocytes. As a rule, both the cytoplasm and the chromatin of the parasites stained fully as well in the thick as in the corresponding thin smear, or even better. We found that much depends on the character of the water used in diluting the stain. Spring or tap water laked out the blood well, but the parasites sometimes failed to take a good stain. Distilled water without the addition of some alkali often stained the background too red. We got our best results with rain water or a mixture of rain water with distilled water. Bacteria in water, especially from the bottom of a container, sometimes occur in numbers sufficient to obscure the background, and sometimes animalcules breed in water which might possibly be confused with some blood parasites. To avoid these we usually autoclaved the fresh or comparatively bacteria-free rain or distilled water and kept it in bottles ready for use. Chromatin granules, or red chromatinlike granules, not associated with the blue cytoplasm were never counted as parasites.

The thick-film method in the examination of blood for parasites,

which was introduced by Ross [8] and employed by Koch [9] in the examination for trypanosomes, has found favor with many investigators of malaria. It was modified by Ruge [10] and employed by Dempwolf.[11]

Dempwolf reports on malaria examinations in Daressalam, in which examinations by the thick and the thin smears are compared. Summarizing all cases, which include children and adults, quinine treated and untreated, and various nationalities, 9,758 examinations by the thin-smear method gave 8.5 per cent positive, while 5,770 examinations by the thick-film method gave 25.7 per cent positive. In this method the unfixed thick film was dried for from two to twenty-four hours, then a mixture of 2 drops of Giemsa stain to 2 cubic centimeters of water was poured on the slide and allowed to remain for from fifteen to twenty minutes. The slide was then rinsed and dried. The author recommends the thick-film method for the examination for blood parasites where large numbers of persons must be examined.

James [12] made use of the thick-film method. He first laked out the hæmoglobin in ethyl alcohol plus a small percentage of hydrochloric acid (10 drops of commercial hydrochloric acid to 50 cubic centimeters of alcohol) fixed to the slide, washed the slide for from ten to fifteen minutes in running tap water, dried it in the air, and then stained with any good modification of the Romanowski method, such as Hastings's, Wright's, or Leishman's. The stain in liberal quantity was put on the slide, allowed to remain two or three minutes to fix, then diluted with all the distilled water that the slide would hold. After a few minutes he diluted again and after five minutes often made a further dilution. The stained slide was washed in tap water. In 100 cases of malaria in the Canal Zone he obtained 94 per cent positive by the thick-film method where the thin-film method of the same cases gave only 60 per cent positive. The time of examination was limited to five minutes for the thick films from each patient. By the thick-film method he was able to demonstrate parasites in quinine-treated cases, on the average, three days after the beginning of the treatment, even in latent or scanty infections.

[8] *Rept. Thompson Yates Lab.* (1903), **5**, part I.
[9] *Deutsch. Med. Wochenschr.* (1907), 1889.
[10] Malariakrankheiten, 2 Aufl. (1906), 290.
[11] *Arch. f. Schiffs- u. Tropenhyg.* (1908), **12**, 435.
[12] *Proc. Canal Zone Med. Assoc.* (April to September, 1911), **4**, pt. I, 49.

Muehlens,[13] in an investigation of malaria in Emden, Germany, used the thick-film dried and stained unfixed in Giemsa's stain in the usual dilution. He compared the thick- and thin-film method by examining for the same period of time (at first ten then five minutes) thick and thin preparations from each person. He found both thick and thin positive one hundred thirty-one times; and the thick positive, but the corresponding thin negative, one hundred two times.

Masterman [14] successfully used the thick-film method in the investigation of latent malaria in Palestine. He used the method of Muehlens.

In our own work, dealing largely with latent malaria in which the parasites are often very few, we have found the thick-film method invaluable. In many preparations a parasite will be found only after many fields of the thick film have been searched, and in a comparatively small percentage of our cases only a single ring, plasmodium, or crescent could be found in the entire preparation. When a parasite was found in these scanty infections, we made it a rule to search for other parasites for confirmation unless the character of the first one found also was beyond doubt. Doubtful parasites are sometimes found in thin preparations as well as thick, and the thick offers the advantage of giving a better chance of finding a second parasite for confirmation.

In some preparations a part of the red corpuscles containing malarial parasites failed to lake out and stood out conspicuously against the background, so that they could readily be found and their species determined. This behavior of the parasitized corpuscle, by no means constant, was noted in both tertian and æstivo-autumnal infections.

A matter of much importance in the examination of blood smears for malarial parasites is the time necessary to give to the examination of a preparation before declaring it negative. In our work, where thousands of preparations had to be examined by only two persons, a saving of time and eye strain was of vital importance.

In all the preparations from three towns, and in the greater part of those from a fourth, we carefully noted the time given to examination before a preparation was declared negative, and we also noted the time required to find the first parasite in the

[13] *Beih. z. Arch. f. Schiffs- u. Tropenhyg.* (1912), 16, 46.
[14] *Journ. Hyg.* (1913), 13, 49.

positives. A large proportion of positives from these towns were latent cases, and the number of parasites found per slide was small. Of the total 75 positives only 4 preparations showed as many as 1 parasite per leucocyte. The time given to examinations in these towns is shown in Table V.

TABLE V.—*Time devoted to examination of preparations from Mabitac, Santa Maria, Lucena, Tayabas, and the greater part of those of Siniloan. Thick films.*

Negative.		Positive.	
Time examined before the preparation was declared negative.	Slides examined of which time was taken.	Time required to find the first parasite.	Slides examined of which time was taken.
4 minutes	9	Immediately	13
4.5 to 4.75 minutes	23	5 to 10 seconds	4
5 minutes	236	15 to 25 seconds	8
5.25 to 5.75 minutes	48	30 to 45 seconds	13
6 minutes	73	1 minute	6
6.25 to 6.75 minutes	24	1.2 to 1.5 minutes	10
7 minutes	19	2 minutes	3
7.25 to 7.75 minutes	8	2.25 to 2.5 minutes	6
8 minutes	9	3 minutes	2
8.25 to 8.75 minutes	1	3.25 to 3.5 minutes	3
9 minutes	4	4 minutes	3
9.5 minutes	1	4.5 minutes	1
10 minutes	4	5.5 minutes	1
10.5 minutes	1	6 minutes	1
13 minutes	1	6.5 minutes	1
15 minutes	2		
Total	463	Total	75

Summary of Table V.

Time required for 463 negative slides	2,588 minutes.
Average time per negative slide	5.6 minutes.
Total number of negatives and positives	538
Percentage of positives	13.9

There were 538 preparations of which the time was taken. Seventy-five, or 13.9 per cent, were positive, and the average time of examination of negatives was 5.6 minutes. This may be slightly in excess of the average time in all our examinations, but had the time of examination for the series in Table V been limited to four minutes, we would still have had 13.1 per cent positive. If it had been limited to even three minutes, we would have had 11.7 per cent positive. So it is evident that the percentage of error is small for the time we actually devoted to examination in our series. It must, also, be taken into consid-

eration that positives often show something suspicious (basophilic erythrocytes, granules of chromatin, or the like), so that the search for parasites in such slides is prolonged beyond the average time. In the course of antimalarial work it might be necessary to make a malarial survey in the shortest possible time. In such a case an experienced worker might limit the time devoted to each thick film to three minutes or less and obtain results sufficiently accurate for practical purposes. The thick films were so spread as to present thicker and thinner areas, and both sorts were included in the search.

Nearly all of the spleen examinations were made by Raquel and Guzman, but those of the third examination of Magdalena, those of Cagayan Province, and a few others were made by Rosa. While as a routine the blood specimens were taken at the same time that the spleen examination was made, our records for some localities show more spleen examinations than blood examinations. In most cases this is due to the fact that the slides were unfit for examination, usually because we were unable to get at them before the hæmoglobin had become too dry to lake out well. Many of the cases occurred during our absence in Mindoro and when the weather was hot and dry.

The general result of the malarial survey is given by provinces in Table VI, together with some notes on the anopheles survey of each locality. Nearly all of the mosquito surveys were done by Barber. A large proportion of the localities were visited, but it was impracticable to visit all of them in the time at our disposal. Some of them, as those in Cagayan Province, would require a journey of about three weeks.

In choosing localities for survey we aimed, first, to include localities of various topographical types as far as possible representative of the main centers of population of the Archipelago; secondly, to survey some localities known to be malarious in order to determine why and to what extent the disease is prevalent in them. In the Philippines nearly all the population is found in towns or villages; few people live in scattered houses; so the indexes of towns fairly represent that of the whole population. Where the word "town" is used in this paper it has reference to the center of population, and does not include the surrounding country. In the case of school children the surrounding country is in a measure represented by children temporarily residing in the town. All dates are 1914 unless otherwise stated.

TABLE VI.—*Descriptions of malaria and Anopheles surveys.*

Tracing No.	Locality.	Province.	General topography.	Anopheles-mosquito survey (by Barber).
1	Manila, districts of Malate and Tondo.		Low coast; swampy esteros and fishponds containing brackish water; no fresh brook or stream near.	Dry and rainy season, *A. rossii* numerous in algæ in fishponds and in esteros; children for examination were selected from among those living nearest the breeding places of *A. rossii.*
2	Calamba (first examination)	Laguna	Plain; town surrounded by rice paddies irrigated the year around.	Numerous visits, March to October. *A. rossii* numerous in neighboring rice paddies and in the river, few in ditches in the streets; *A. barbirostris* in river.
2a	Calamba (second examination)	do	River one side of the town	
3	Biñan	do	Plain; rice paddy and cane region, long cultivated, irrigated; brook near town.	
4	San Pedro Tunasan	do	Plain; rice paddy and cane region; long cultivated; brook near town.	
5	Canlubang	do	Plain, somewhat rolling; brook near town, sugar cane plantation recently opened.	December, 1913, *A. maculatus* in brook; January, 1914, to August, 1914, numerous *A. febrifer* and some *A. barbirostris* in brook; *A. rossii* in this brook and pools near the town.
6	Suliaw, barrio of Canlubang	do	Plain; similar to Canlubang; same locality	Same species as at Canlubang.
7	Siniloan (first examination)	do	Plain; ditches from river on both sides of the town and a brook through it.	Apr. 22, *A. febrifer* numerous in ditch of clear water at edge of the town, *A. barbirostris* in the ditches; no *Anopheles* in the sluggish brook; short search.
7a	Siniloan (second examination)	do		
8	Santa Maria (first examination)	do	Hilly; river close by town	Apr. 22, *A. febrifer* in grassy edge of river near town.
8	Santa Maria (second examination)	do		

TABLE VI.—*Descriptions of malaria and Anopheles surveys*—Continued.

Tracing No.	Locality.	Province.	General topography.	Anopheles-mosquito survey (by Barber).
9	Lilio (first examination)	Laguna	Hilly; numerous ditches of clear water in streets; brook beside town; coconut region.	Apr. 18 and Oct. 24, *A. febrifer* in ditches in streets, also in brook and in crevices of ditch wall near houses.
9a	Lilio (second examination)	do	do	
10	Magdalena (first examination)	do	Plain; numerous fresh-water canals in town; a brook on either side of town; swampy near market and toward lower end of town; coconut region.	
10a	Magdalena (second examination)	do		Apr. 17, numerous *A. febrifer* in ditches in streets; also found July 31, Aug. 19, and Oct. 23; *A. febrifer* in brooks not numerous; *A. rossii* in pools near new market, and *A. sinensis* (?) in a ditch in a street.
10b	Magdalena (third examination)	do		
11	Balian	do	Hilly	
12	Los Baños (first examination)	do	Hilly; on Laguna de Bay; brooks in region, but none in or very near the town.	Mar. 22, *A. rossii* numerous in algæ in the lake, also in pools and thermal springs at edge of lake next to the town.
12a	Los Baños (second examination)	do	Hilly	
13	Los Baños, College of Agriculture	do	Mountain side; wood, but little undergrowth near college; brook near dormitories.	Mar. 22, *A. febrifer* few in brook near dormitories.
14	Mabitak (first examination)	do	Hilly; river immediately by town; conditions similar to those of Santa Maria, Laguna.	Apr. 22, few *A. febrifer* in river by town.
14a	Mabitak (second examination)	do	do	
15	Majayjay (first examination)	do	Hilly near high mountain; numerous ditches in streets; jungly brooks in and near town.	Apr. 17, *A. febrifer* in ditch in street and in brook; Oct. 23, *A. febrifer* in street ditches and numerous in brook in town.

No.	Town	Province	Description	Remarks
15a	Majayjay (second examination)	--do--	Hilly; near high mountain; numerous ditches in streets; jungly brooks in and near town.	
16	Nagcarlan (first examination)	--do--	Hilly; numerous ditches in streets, and jungly brooks in and near town.	Apr. 18, *A. febrifer* in brook; Oct. 24, *A. febrifer* in ditches in streets and jungly brook at edge of town.
16a	Nagcarlan (second examination)	--do--	...do...	
17	Pakit	--do--	Hilly; near mountains.	
18	Paete	--do--	Hilly; near mountains; ditch of very swift water through town.	
19	Pangil	--do--	Hilly	
20	Parañaque	Rizal	Low coast with esteros	Numerous dates in 1913 and 1914; *A. rossii* very numerous in an estero near Santa Monica.
21	Santa Monica, barrio of Parañaque	--do--	Low coast with esteros full of algæ	
22	San Mateo	--do--	Plain, rice-paddy region; river with broad open bed near town.	Oct. 21, a few *A. rossii* in a pool near river; no other species found.
23	Guinayang, barrio of San Mateo	--do--	Plain, rice-paddy region; small brook through barrio; larger near barrio.	Oct. 21, no larvæ found in either brook during a short search.
24	Montalban	--do--	Plain near hills; river with broad open bed at edge of town; wooded brook through the end of town.	Oct. 21, *A. febrifer* in brook, not numerous.
25	Antipolo	--do--	In mountains, about 200 meters' elevation; several brooks in town, but most houses well above them.	Aug. 8, *A. febrifer*, *A. maculatus*, and *A. barbirostris* found in brooks, in town.
26	Cabanatuan	Nueva Ecija	Plain; large river at side of town; brook through edge of town.	December, *A. febrifer* few in brook in town; no *Anopheles* found after short search in river; *A. rossii* in overflow from artesian well.
27	Aduas, barrio of Cabanatuan	--do--	Plain.	
28	Valdefuente, barrio of Cabanatuan [a]	--do--	Plain, near large river; country somewhat wooded.	Dec. 31, incomplete survey; no *Anopheles* found in river; no brook at present, after 3 months of drought.
29	Gapan	--do--	Level plain, at side of river with broad open bed; country open; rice, corn, and cane; barrio across river said to be malarious.	Jan. 1, 1915, *A. rossii* in overflow of artesian well, *A. rossii* and *A. barbirostris* in stagnant water of old bed of river.
30	Santa Rosa	--do--	Level plain; country open; mostly rice culture.	

[a] Some selection of sick, including one positive.

TABLE VI.—*Descriptions of malaria and Anopheles surveys*—Continued.

Trac-ing No.	Locality.	Province.	General topography.	Anopheles-mosquito survey (by Barber).
31	San Isidro	Nueva Ecija	Level plain: large river with broad open bed; country open; rice, corn, and cane.	Jan. 1, 1915, no *Anopheles* larvæ found in river or elsewhere in town.
32	Bongabon	...do	Plain, near mountains; canal from river directly through town and very near houses; town overgrown, and much jungle in surrounding country.	Dec. 30, *A. febrifer* very numerous in canal; no other species found there.
33	Viga Grande, barrio of Bongabon	...do	Plain, near mountains; irrigation ditch near town; country with much high grass and trees.	Dec. 30, *A. febrifer* and *A. barbirostris* in irrigation ditch.
34	Makapsing, barrio of Bongabon	...do	Plain, near mountains; brook by barrio; high-grass region.	Dec. 30, *A. febrifer* and *A. barbirostris* in brook near town.
35	San Esteban, barrio of Bongabon	...do	Plain, near mountains	Dec. 30, *A. febrifer* in brook.
36	Santop, barrio of Bongabon	...do	Plain, near mountains; large brook edge of town; surrounding country mostly wooded, with some rice fields.	
37	Rizal	...do	Plain, near mountains; large irrigation ditch through town; small brook at edge of town; surrounding country with woods and grass.	Dec. 30, *A. febrifer* (possibly *maculatus*), in irrigation ditch and brook; *A. barbirostris* in brook.
38	Abulug	Cagayan	On river	
39	Maura, barrio of Aparri	...do		
40	Iguig	...do	Hilly	
41	Ballesteros	...do		
42	Camalañugan	...do	River bank	
43	Alilim	...do		
44	Tuguegarao	...do	Valley	
45	Nasugbo	Batangas		
46	Atimonan	Tayabas	Coast	

47	Lucena	do	Plain near coast; large river and smaller one at edge of town; tide water into larger river.	Jan. 8, 1915, *A. rossii* in pool, *A. barbirostris* in smaller river.
48	Lucban	do	Mountain; ditches of flowing water in streets; brooks in town.	Jan. 7, 1915, *A. febrifer* in brook, none in ditches; *A. barbirostris* in water seeping from ditch.
49	Tayabas	do	Hilly; numerous ditches with clear water in streets; brook at side of town.	Nov. 14, *A. febrifer* in ditches in streets.
50	Sariaya	do	Hilly; ditches with flowing water in streets; coconut region.	Jan. 8, 1915, *A. febrifer* in ditch in street; found only in one locality after considerable search.
51	Camp Stotsenberg, Filipino barrio (first examination.)	Pampanga	Plain, sandy, near mountain; clear brook immediately by barrio.	July, *A. maculatus* and *A. febrifer* in brook, very near houses.
51a	Camp Stotsenberg, Filipino barrio	do	do	
52	Hagonoy	Bulacan	Low plain near coast.	
53	Pulilan	do	Plain.	
54	Dampol, barrio of Pulilan	do	do	
55	Baliuag	do	Plain; nonirregated rice region.	
56	San Jose, barrio of Baliuag	do	Plain	
57	Sabang, barrio of Baliuag	do	do	
58	Malolos	do	do	
59	Matimbo, barrio of Malolos	do	do	
60	Santo Cristo, barrio of Malolos	do	do	
61	Paombong	do	Plain; large river by town.	
62	Limay	Bataan	Low coast; brook and rice paddies above town.	Aug. 12, recent freshet; *A. febrifer* (probably) in brook.
63	Orion	do	Low coast; rice paddy region	Aug. 12, *A. rossii* in rice paddies and in basin below artesian well in street.
64	Olongapo	Zambales	Coast.	
65	Cebu	Cebu	Low coast; esteros.	Sept. 10, *A. rossii* numerous in brackish pool near prison, also in overflow from well of fresh water.
66	Bolhoon	do	Low coast; brook about 0.5 kilometer from town; ditch with clear water in streets; ditches between 100 and 200 meters long; coconut region.	Sept. 13, *A. febrifer* in brook; *A. febrifer*, *A. rossii*, and *A. barbirostris* in ditch.

TABLE VI.—*Descriptions of malaria and Anopheles surveys*—Continued.

Tracing No.	Locality.	Province.	General topography.	Anopheles-mosquito survey (by Barber).
67	Argao	Cebu	Coast; brook at edge of town	Sept. 14, *A. febrifer* (few) in brook; *A. rossii* in brackish pool near town.
58	Carcar	do	Coast; brook near town	Sept. 15, *A. febrifer* (few) in brook.
69	San Jose, including barrio b	Mindoro	Plain; brook, irrigation ditches, and large river. (See Table I and text.)	May 1 to May 31, *A. febrifer* in brook, river, and ditches; *A. maculatus* in ditches; *A. rossii* in swamp near brook.
70	Mangarin b	do	Low coast; no flowing water near except spring in town during wet season.	May 2, *A. rossii* in brackish pool near houses; May 21, *A. febrifer* very numerous in river 1 to 2 kilometers from town; August, *A. febrifer* caught in houses in town.
71	Iwahig	Palawan	Plain near coast; river at edge of central barrio; brooks at various parts of the estate; surroundings, woods and coconut plantations.	June, *A. febrifer* numerous in river and in various brooks; also *A. rossii* and *A. barbirostris*; *A. febrifer* (numerous), *A. rossii*, *A. barbirostris* (few) caught in houses.
72	Puerto Princesa	do	High coast; ditch fed by springs at edge of town.	June 21, *A. febrifer* numerous in limited breeding place in ditch at edge of town; *A. rossii* in the same locality.

b In a malarial survey of the San Jose Estate made January, 1912, Walker, Guzman, and Concepcion [*This Journal, Sec. B* (1914), 9, 187] obtained the following results: Well men, 978 examined, 34.05 per cent positive; well women, 63 examined, 22.22 per cent positive; well children, 23 examined, 43.48 per cent positive. In the same survey Musgrave found a spleen index of 98 among the children of Mangarin.

TABLE VI.—*Descriptions of malaria and Anopheles surveys*—Continued.

Trac-ing No.	Date of visit	Age	Malaria survey. Number.	Parasites positive. Number.	Per cent.	Enlarged spleen. Number.	Per cent.	Parasites positive.c	Examiner. Blood.	Spleen.
	1914.									
1	Summer	1 to 10 years d	293	0	0.0	0	0.0		Barber and Guzman	Raquel.
		11 to 15 years d	3	0	0.0	0	0.0			
		1 to 10 years e	216	0	0.0	0	0.0			
		11 to 15 years e	8	0	0.0	0	0.0	0.0		
		Total	520	0	0.0	0	0.0			
		3 to 10 years f	221							
2	March 18	3 to 10 years	45	0	0.0			0.0	Barber	
		11 to 12 years	7	0	0.0			0.0		
		Total	52	0	0.0					
2a	August 6	1 to 10 years	116	0	0.0	0	0.0	0.0	Barber and Guzman	Do.
3	March 22	6 to 10 years	36	0	0.0			0.0	Walker	
4	March 24	7 to 10 years	18	0	0.0			0.0	do	
5	January 22	6 to 10 years	34	2	5.9			100.0	Barber	
		11 to 15 years	9	0	0.0					
		Total	43	2	4.6					
6	July 7	1 to 7 years	11	0	0.0	6	55.5	0.0	do	Do.

c Percentage of parasites positive where the number of parasites equaled or exceeded 1 leucocyte.
d Malate.
e Tondo.
f Malate (spleen only examined).

TABLE VI.—*Descriptions of malaria and Anopheles surveys*—Continued.

Tracing No.	Date of visit	Age	Malaria survey						Examiner	
			Number.	Parasites positive.		Enlarged spleen.		Parasites positive.	Blood.	Spleen.
				Number.	Per cent.	Number.	Per cent.			
7	July 10	1 to 10 years	57	5	8.7	27	47.3		Barber	Raquel.
		11 to 12 years	2	0	0.0	1	50.0			
		Total	59	5	8.4	28	47.4	0.0		
		1 to 10 years g	53			19	35.8			
		11 to 15 years g	2			1	* 50.0			
		Total	55			20	34.5			
		Total of spleen	114			48	46.1			
7a	December 2	5 to 10 years	103	8	7.8	13	12.6		Barber and Guzman	Guzman.
		11 to 13 years	7	3	42.9	2	28.6			
		Total	110	11	10.0	15	13.6	0.0		
8	May 9	1 to 10 years	34	8	23.5	24	70.6		do	Raquel.
		11 years	1	0	0.0	1	100.0			
		Total	35	8	22.7	25	71.4	35.0		
8a	December 4	1 to 10 years	36	13	36.1	16	44.4		Barber	Guzman.
		11 to 14 years	7	3	42.9	1	14.3			
		Total	43	16	37.2	17	39.5	6.3		
9	April 30	2 to 10 years	45	0	0.0	3	6.6		do	Raquel.
		11 to 13	9	0	0.0	1	11.1			
		Total	54	0	0.0	4	7.4	0.0		

No.	Date	Age group	Number examined	Parasite positive, no.	Parasite positive, per cent	Spleen positive, no.	Spleen positive, per cent	Per cent[c]	Observer
9a	August 19	7 to 10 years	50	7	14.0	2	4.0		Do.
			g 70			0	0.0		do
		Total	120			2	1.7		do
10	March 28	6 to 10 years	85	16	18.8				Do.
		11 to 18 years	52	10	19.5				do
		Total	137	26	19.0			4.1	do
10a	July 22	6 to 10 years	92	58	63.0	46	51.1		Do.
		11 to 18 years	36	16	44.4	4	11.1		do
		Total	128	74	57.8	50	39.1	9.9	do
10b	October and November.	6 months and under	11	2	18.2				Rosa.
		do	4			0	0.0		do
		7 to 11 months	11	6	55.6				
		do	10			4	40.0		
		1 to 5 years	113	79	69.9				
		do	107			76	71.0		
		6 to 10 years	170	81	47.7				
		do	139			102	73.4		
		11 to 15 years	66	27	40.9				
		do	32			25	78.1	11.9	
		Total parasite	371	195	52.6				
		Total spleen	292			207	70.9		
11	May 4	1 to 10 years	30	1	3.3	2	6.6	0.0	Raquel.
12	March 19	5 to 10 years	16	0	0.0	0	0.0	0.0	do / do
12a	August 6	5 to 10 years	96	6	6.3	0	0.0		Barber and Guzman.
		11 to 12 years	1	1	100.0	0	0.0		Do.
		Total	97	7	7.2	0	0.0	14.3	
13	August 7	Adults	50	1	2.0	0	0.0	0.0	Do.

c Percentage of parasites positive where the number of parasites equaled or exceeded 1 leucocyte.

g Spleen only examined.

TABLE VI.—*Descriptions of malaria and Anopheles surveys—Continued.*

Tracing No.	Date of visit.	Age.	Malaria survey.							Examiner.	
			Number.	Parasites positive.		Enlarged spleen.		Parasites positive.c	Blood.	Spleen.
				Number.	Per cent.	Number.	Per cent.			
	1914.									
14	May 8	1 to 10 years	20	0	0.0	1	5.0	0.0	Barber	Raquel.
		do	ᵍ34			10	29.4			
		11 to 12 years	ᵍ6			1	16.7			
		Total	60			12	20.0			
14a	December 3	4 to 10 years	83	7	8.4	13	15.6		do	Guzman.
		11 to 15 years	8	2	25.0	1	12.5			
		Total	91	9	9.9	14	15.4	22.2		
15	March 31	6 to 10 years	47	3	6.4				do	
		11 to 15 years	2	1	50.0					
		Total	49	4	8.2			0.0		
15a	August 18	2 to 10 years	51	18	35.3	46	90.2	16.7	do	Raquel.
		do	ᵍ67			2	2.9			
		Total	118			48	40.7			
16	April 29	1 to 10 years	43	5	11.5	3	7.0		do	Do.
		11 to 15 years	10	1	10.0	2	20.0			
		Total	53	6	11.5	5	9.4	16.7		

No.	Date	Age	Number examined	Positive (no.)	Per cent	Positive (no.)	Per cent	Per cent [a]	Observer	
16a	August 20	7 to 10 years	50	15	30.0	10	20.0	0.0		Do.
		11 to 13 years	2	0	0.0	0	0.0		do	
		Total	52	15	28.9	10	19.5	0.0		
17	May 3	Spleen only	60			0	0.0			Do.
		Total	112			10	8.9			
		1 to 10 years	98			11	11.8			
18	May 2	do	108			2	1.9			Do.
		11 to 12 years	2			0	0.0			
		Total	106			2	1.9			
19	May 6	1 to 10 years	57			28	49.1			Do.
20	August 14	6 to 10 years	101			0	0.0		Guzman	Do.
21	April 27	1 to 10 years	21	0	0.0	0	0.0			Do.
		11 to 13 years	4	0	0.0	0	0.0		Barber	
		Total	25	0	0.0	0	0.0	0.0		
22	October 15	7 to 10 years	110	1	0.9	9	8.2			Guzman.
		11 to 15 years	59	0	0.0	4	6.7		Guzman	
		Total	169	1	0.5	13	8.9	0.0		
23	do	7 to 10 years	22	1	4.5	1	4.5			Do.
		11 to 16 years	23	0	0.0	3	13.0		do	
		Total	45	1	2.2	4	8.8	100.0		
24	October 16	7 to 10 years	34	1	2.9	1	2.9			Do.
		11 to 13 years	21	0	1.0	0	0.0		do	
		Total	55	1	1.8	1	1.8	0.0		
25	August 12	6 to 10 years	123	2	1.6	0	0.0	0.0	Barber and Guzman	Raquel.

[c] Percentage of parasites positive where the number of parasites equaled or exceeded 1 leucocyte.

[g] Spleen only examined.

TABLE VI.—Descriptions of malaria and Anopheles surveys—Continued.

Tracing No.	Date of visit.	Age.	Malaria survey.					Parasites positive.c	Examiner.	
			Number.	Parasites positive.		Enlarged spleen.			Blood.	Spleen.
				Number.	Per cent.	Number.	Per cent.			
26	November 9	5 to 10 years	156	5	3.2	21	18.4		Guzman	Guzman.
		11 to 13 years	17	1	5.8	0	0.0			
		Total	173	6	3.5	21	12.1	16.7		
27	November 10	5 to 10 years	62	1	0.0	8	12.7		do	Do.
		11 to 14 years	11	0	0.0	1	9.0			
		Total	73	0	0.0	9	12.3			
28	do	2 to 10 years	41	6	14.6	14	34.2		do	Do.
		11 to 15 years	10	3	30.0	4	40.0			
		Total	51	9	17.6	18	35.3	22.2		
29	November 13	5 to 10 years	108	0	0.0	11	10.2		do	Do.
		11 to 13 years	6	0	0.0	0	0.0			
		Total	114	0	0.0	11	9.6			
30	November 11	5 to 10 years	112	2	1.8	15	13.4		do	Do.
		11 to 13 years	7	0	0.0	0	0.0			
		Total	119	2	1.6	15	12.6	50.0		
31	November 12	4 to 10 years	117	2	1.7	9	7.6		do	Do.
		11 to 15 years	22	0	0.0	1	4.8			
		Total	139	2	1.4	10	7.2	0.0		

c Percentage of parasites positive where the number of parasites equaled or exceeded 1 leucocyte.

No.	Date	Age group							Collector	Locality
32	November 18	5 to 10 years	58	14	24.1	24	41.4			
		11 to 21	68	19	26.5	17	25.0			
		Total	126	33	26.2	41	32.5	3.0	do	Do.
33	November 19	7 to 10 years	25	6	24.0	15	60.0			
		11 to 15 years	8	1	12.5	4	50.0			
		Total	33	7	21.2	19	54.6	14.3	do	Do.
34	do	3 to 10 years	38	5	13.2	12	31.6		Barber	
		11 to 14 years	5	0	0.0	2	40.0			
		Total	43	5	11.6	14	32.7	0.0		Do.
35	November 18	5 to 10 years	18	7	38.9	10	55.6		Guzman	
		11 to 15 years	12	2	16.6	7	58.3			
		Total	30	9	30.0	17	56.7	0.0		Do.
36	do	4 to 10 years	24	3	12.5	10	41.7		do	
		11 to 15 years	10	2	20.0	6	60.0			
		Total	34	5	14.7	16	47.1	0.0		Do.
37	November 19	5 to 10 years	27	2	7.4	4	14.8		Barber and Guzman	
		11 to 13 years	13	1	7.7	4	30.8			
		Total	40	3	7.5	8	20.0	0.0		Do.
38	September 7	8 to 10 years	17	0	0.0	7	41.2		Guzman	
		11 to 13 years	5	1	20.0	4	80.0			
		Total	22	1	4.5	11	50.0			Rosa.
39	September 8	10 to 19 years	30	2	6.7	14	46.7	100.0	do	Do.
40	September 4	1 to 14 years	15	0	0.0		0.0	50.0	do	
41	September 7	6 to 10 years	5			3	60.0			
		11 to 18 years	11			6	54.5			
		Total	16			9	56.3			Do.

TABLE VI.—Descriptions of malaria and Anopheles surveys—Continued.

Tracing No.	Date of visit.	Age.	Malaria survey.						Examiner.	
			Number.	Parasites positive.		Enlarged spleen.		Parasites positive.[c]	Blood.	Spleen.
				Number.	Per cent.	Number.	Per cent.			
	1914.									
42	September 9	8 to 10 years	5			3	60.0		Rosa.	
		11 to 15 years	12			4	33.3			
		Total	17			7	41.2			
43	do	6 to 10 years	6			2	33.3			Do.
		11 to 14 years	8			5	62.5			
		Total	14			7	50.0			
44	September 3	10 to 16 years	16	5	31.3	8	50.0	0.0	Guzman	
45		9 to 15 years	20	0	0.0	5	25.0			Do.
46	December 10	6 to 10 years	20			6	30.0	0.0		Do.
47	December 9	5 to 10 years	69	3	4.3	7	10.1		Barber and Guzman	Guzman.
		11 to 14 years	36	1	2.8	2	5.6			
		Total	105	4	3.8	9	8.6	0.0		
48	December 11	5 to 10 years	108	4	3.7	16	14.8		do	Do.
		11 to 13 years	4	0	0.0	0	0.0			
		Total	112	4	3.6	16	14.4	0.0		
49	December 10	5 to 10 years	76	23	30.3	21	27.9		do	Do.
		6 to 15 years	34	9	26.5	15	44.1			
		Total	110	32	29.1	36	32.7	3.1		

[c] Percentage of parasites positive where the number of parasites equaled or exceeded 1 leucocyte.

No.	Date	Age group	Number examined	Positive	Per cent	Positive	Per cent	Per cent	Examiner	Locality
50	1915. January 8	6 to 10 years	29	1	3.5				Barber	Luis Guerrero.
		11 to 15 years	16	1	6.7					
		Total	45	2	4.4					
	1914.	0 to 15 years	29			15	51.7			
51	May 16	5 to 10 years	22	5	22.7	9	40.9		do	Raquel.
		11 to 13 years	9	2	22.2	2	22.2			
		Total	31	7	22.6	11	35.5	0.0		
51a	August 28	6 to 10 years	46	7	15.2	8	17.4		do	Do.
		11 to 15 years	24	3	12.5	3	12.5			
		Total	70	10	14.3	11	15.7	10.0		
52	October 23	6 to 10 years	101			4	3.9			Guzman.
		11 to 15 years	43			4	9.4			
		Total	144			8	5.6			
53	October 27	6 to 10 years	70	0	0.0	0	0.0		Barber and Guzman	Do.
		11 to 15 years	28	0	0.0	0	0.0			
		Total	98	0	0.0	0	0.0	0.0		
54	October 28	6 to 10 years	85			5	5.8			Do.
		11 to 13 years	30			1	5.3			
		Total	115			6	5.2			
55	October 27	6 to 10 years	157			6	3.8			Do.
		11 to 14 years	60			2	3.3			
		Total	217			8	3.7			
56	do	6 to 10 years	35			1	2.9			Do.
		11 to 13 years	9			0	0.0			
		Total	44			1	2.3			

TABLE VI.—*Descriptions of malaria and Anopheles surveys*—Continued.

Tracing No.	Date of visit.	Age.	Malaria survey.						Examiner.	
			Number.	Parasites positive.		Enlarged spleen.		Parasites positive.[c]	Blood.	Spleen.
				Number.	Per cent.	Number.	Per cent.			
	1914.									
57	October 26	2 to 10 years	41			3	7.3			Guzman.
		11 to 13 years	12			0	0.0			
		Total	53			3	5.7			
58	October 20, 21	5 to 10 years	82			0	0.0			Do.
		11 to 12 years	23			0	0.0			
		Total	105			0	0.0			
59	October 22	6 to 10 years	64			0	0.0			Do.
		11 to 15 years	1			0	0.0			
		Total	65			0	0.0			
60	do.	5 to 10 years	76			0	0.0			Do.
		11 to 15 years	22			0	0.0			
		Total	98			0	0.0			
61	October 22, 23	6 to 10 years	74			0	0.0			Do.
		11 to 14 years	54			1	1.8			
		Total	128			1	0.8			
62	September 7	7 to 10 years	44	3	6.8	1	2.3	100.0	Guzman	Raquel.
63	September 9	6 to 10 years	50	0	0.0	0	0.0	0.0	do	Do.

[c] Percentage of parasites positive where the number of parasites equaled or exceeded 1 leucocyte.

No.	Date	Age	Examined	Positive	Per cent	No.	Per cent	Per cent	Observer	Remarks
64	May 26	2 to 10 years	42	0	0.0	7	16.7		Barber	Do.
		11 to 12 years	5	0	0.0	0	0.0			
65		Total	47	0	0.0	7	14.8			
66	September 17	6 to 10 years	40	0	0.0	9	22.5	0.0	Barber	Do.
67	September 15, 16	4 to 10 years	100	0	0.0	2	2.0	0.0	Barber and Guzman	Do.
68	September 18	4 to 10 years	100	0	0.0	6	6.0	0.0	do	Do.
69	May 3 to 25	10 years and under	16	8	50.0				Barber	
		10 to 15 years	16	4	25.0					
		16 years and over	185	69	37.3					
		Total	217	81	37.3			11.4		
70	May 25	2 to 10 years	21	9	42.9				do	
		11 to 14 years	7	2	28.6					
		Total	28	11	39.3			0.0		
71	June 23	Adults	62	8	12.9				do	
72	June 21	7 months to 10 years	21	7	33.3				do	
		11 to 14 years	7	1	14.3					
		Total	28	8	28.6			0.0		

Summary of Table VI.

	Age.	Exami-nations.	Parasites positive.		Spleen positive.	
			Number.	Per cent.	Number.	Per cent.
All ages:						
Both spleen and parasites		4,360				
Spleen only		1,844				
Parasites only		795				
Total		6,999				
Total examinations for parasites.		5,155				
Persons examined, all ages (deducting for localities surveyed twice or three times), estimated:						
Both spleen and parasites		3,928				
Spleen only		1,598				
Parasites only		725				
Total		6,251				
Total persons examined for parasites.		4,653				
Children only, parasites	10 years and under.	4,051	466	11.5		
	11 to 15 years	695	115	16.6		
Total		4,746	681	14.3		
Children only, spleen	10 years and under.	5,046			755	15.0
	11 to 15 years	855			125	14.6
Total		5,901			880	14.9
Children only, one survey of each locality included, thus eliminating all duplicate examinations.	10 years and under.	3,545	366	10.3		
	11 to 15 years	568	85	15.0		
Total		4,113	451	11.0		
Children only, one survey of each locality included, thus eliminating all duplicate examinations.	10 years and under.	4,646			613	13.2
	11 to 15 years	780			113	14.5
Total		5,426			726	13.3

In summarizing the data given in Table VI, we find a parasite index of 11 where only one survey of each locality is included and a spleen index of 13.3. These rates are very probably higher than for children generally in the Philippines, since so many malarious localities were included in our survey. If we omit only one town, Magdalena, from the list, the parasite index drops to 6.8. The spleen index in a general way runs parallel to the parasite index, both in the aggregate and in the separate local-

ities, although in most cases the spleen index is higher. If we make a list which includes only children of whom both blood and spleen were examined, and which includes only one examination of each locality, we have: Examined, 3,686; positive for parasites, 278 or 7.5 per cent; spleen enlarged, 458 or 12.4 per cent. These figures best represent the ratios between the two indexes. In nearly all towns where some positives for parasites were found the spleen index was higher than the parasite index, and many towns show a positive spleen index where the parasite index is 0.

In 314 cases positive for malarial parasites and of whom spleen examinations were also made, 164, or 51.4 per cent, showed an enlarged spleen. Of approximately 569 cases presenting enlarged spleens of whom the blood was examined, 164, or 28.8 per cent, were positive for parasites. It is well known that cases exhibiting splenomegaly, known to be the result of malaria, often fail to show parasites in the blood.

The percentage of parasite-positive cases among spleen-negatives is of comparatively little significance in our series of examinations taken as a whole, since our list includes so large a number of nonmalarious localities. If we include only communities in which at least one parasite-positive was found, we have what is probably a fairer estimate. In these communities approximately 1,883 spleen-negative cases were found of which 144, or 7.7 per cent, were parasite-positive. Only children are included and only those of whom both spleen and blood examinations were made.

The important question comes up as to whether a spleen index of considerable magnitude indicates a present or recent prevalence of malaria in a locality.

Ross, Christophers, and Perry [15] have recently expressed the belief that the spleen rate is the "most readily and extensively applicable, and at the same time the most reliable measure of the amount of malaria in a community" with a serious qualification in the fact "that other diseases than malaria, very notably kala-azar, produce splenic enlargement and may, to an unknown extent, modify or even seriously interfere with the value of the figures obtained." These authors examined 469 children of London and found only about 1 per cent with enlarged spleens. Only about 1 per cent of cases were found in which the question of enlargement was doubtful. They conclude that with the

[15] Proc. Third All-India Sanitary Conf. Lucknow. Jan. 19–27, 1914: Supplement to *Ind. Journ. Med. Res.* (1914), 4, 15.

ordinary palpation as practiced in the field there is small likelihood of serious error.

Gill [16] believes that the parasite rate is of less value than the spleen rate in the measurement of the malaria rate in a community. The number of parasites in the blood may be so small as to be overlooked, they may be reduced by the use of quinine, and the parasite rate varies greatly at different seasons of the year. In northern India he found that benign infections reach their maximum in June, and the subtertian in the autumn. However, while believing that in northern India no definite relationship can be expected to exist between spleen and parasite rates, he found that where the spleen rate is high the parasite rate also tends to be high.

In the Philippines, where there is much less seasonal change than in northern India, we would expect less seasonal variation in the parasite rate. We have found some variation in the parasite rate of towns examined at different times in the same year (Table VI, Nos. 2, 7, 8, 9, 10, 12, 14, 15, 16, and 51), but only one town, Lilio (Table VI, No. 9), showed a negative parasite rate at one examination and a very considerable rate, 14, at another, and in this town the slides were not in perfect condition at the first examination. Many of the towns we examined showed a spleen index of 0, although these towns, while not in a malarious region, were in no way protected from other diseases which might cause enlargement of the spleen. Some towns which show a parasite index very low, even 0, with a high spleen index are in localities where malaria would be expected, as Suliaw and Bolhoon.

While a larger series than ours may be needed to solve this question, we believe that a spleen rate over 10 indicates present or past malaria in the most, if not all, the communities of the Philippines. However, we would not recommend that the malarial survey of a locality should rest on the spleen examination alone.

The amount of enlargement of the spleen was measured in most towns studied. Of 639 examinations where the degree of enlargement of the spleen was reckoned, 336, or 52.6 per cent, were classed as simple palpable; 8, or 1.3 per cent, as 1 centimeter below the costal border; 105, or 16.4 per cent, as 2 centimeters below; 125, or 19.6 per cent, as 3 centimeters below; 22, or 3.4 per cent, as 4 centimeters below, and 43, or 6.7 per cent, as at or below the umbilicus.

[16] Ibid., 18.

Both parasite and spleen indexes of children from 11 to 15 years of age are higher than those of children from 5 to 10 years of age. Possibly if a larger proportion of older children had been included, we would have obtained a different ratio. Again, a very small proportion of children 5 years of age or younger are included. If all children from 1 to 10 years of age had been included, the parasite rate for the younger group probably would have been higher. In the third examination of Magdalena (see Table VI, No. 10) several groups of ages were considered separately. Here we find the highest rate, 69.9 per cent, among children from 1 to 5 years of age.

As a rule, the number of parasites found in positive cases was small, as might be expected in latent malaria. The percentage of positives in which the parasites were approximately as numerous as or exceeded the number of leucocytes is given in Table VI. This gives a rough index of the proportion of more acute cases occurring in a locality. An approximate estimate of the number of parasites was recorded for nearly all positives. For children of 15 years of age and under they are as follows: Only one parasite found after considerable search, 36 cases, or 6.3 per cent; those marked "rare" (about 1 parasite to 10 or 15 fields), 244, or 42.9 per cent; "few" (about 1 parasite to 5 fields), 109, or 19.2 per cent; "+" (1 or more per field), 122, or 21.4 per cent; "++" (1 or more per leucocyte), 58, or 10.2 per cent. All estimates, of course, are only approximate.

As to the type of parasite, quartan occurred more frequently in these latent cases of children than has been our experience in acute cases of adults examined in the tropics. In 272 positives obtained by Walker and Barber in the examination of thin smears from acute cases at Canlubang, 54.4 per cent were subtertian, 41.5 per cent tertian, and only 4.1 per cent quartan.

A frequent finding in the latent cases among children was a very few sporulating quartan. It is sometimes difficult to determine the species of parasite when only a very few can be found in the thick smear. Where plentiful enough they may be found in the thin smear and the identification confirmed. The error is probable in the direction of increase of subtertian at the expense of the other types, where only very scanty numbers of young rings can be found.

Including only persons of 15 years of age and under, results were as follows: Subtertian, 291, or 49.6 per cent; tertian, 185, or 31.6 per cent; quartan, 108, or 18.4 per cent; mixed, 4, or 0.3 per cent. Long search through all preparations would undoubtedly increase the percentage of mixed infections.

As a rule, where considerable numbers of parasites were found, more than one stage of development occurred. This is what would be expected in latent cases, the majority of whom had undoubtedly been infected a long time.

Of 641 positives of 15 years of age or under, 376, or 58.7 per cent, were males and 265, or 41.3 per cent, were females. The town of Magdalena, third examination, gave of 194 males 104, or 53.7 per cent, positive and of 177 females 92, or 52.0 per cent, positive. Examinations of 23 localities including Magdalena gave: Males, 1,360; positive 194, or 14.3 per cent; females, 991; positive, 173, or 17.5 per cent.

While a single survey can give approximately the percentage of persons harboring parasites at any one time, repeated examinations would be necessary to show the number parasitized at some time during childhood, or even during a single year. In the town of Magdalena three surveys were made; the first, March 28; the second, July 22; and the third in late October and early November. About four months intervened between the first and the second and about three months between the second and the third. A record was kept of the name of each person examined. Fifty-eight children came for all three examinations. An analysis of the results of these 58 cases may be of interest (Table VII).

TABLE VII.—*Children of Magdalena examined on three separate occasions.*

	Number.	Per cent.
Positive at all 3 examinations	4	6.9
Positive at 2 examinations, negative at 1	17	29.3
Positive at 1 examination, negative at 2	21	36.2
Negative at all 3 examinations	16	27.6
Total	58	100.0
Positive at first examination	9	15.5
Positive at second examination	35	60.3
Positive at third examination	24	41.4
Positive at at least 1 examination	42	72.4

The highest percentage, 60.3, obtained at the second examination was brought up to 72.4 by the additional positives obtained at the other examinations. It is probable that repeated examinations of the children of this town during two years would give nearly 100 per cent of positives, and that few of the children of this town escape malaria at some time during childhood. None of the 58 of this group were under 6 years of age. A

similar test of children of 5 years of age or under would prob-
ably give higher percentages of positives (Table VI, No. 10,
Magdalena).

The "ditch" or "canal" towns have a peculiar interest on
account of their topography and may well be considered apart.
Water, which is usually obtained from a neighboring brook or
river, is brought into one of these towns by a large ditch or canal.
In the town many laterals carry the water to various streets,
where it is available for laundry or culinary purposes, and
smaller laterals carry it between and under houses for the pur-
pose of carrying away sewage. The water is usually clear, and
may flow swiftly or quietly through the ditches, depending on
the nature of the town site. Small dams are frequently made
in order to obtain a convenient source of water for laundry or
other purposes. The ditches are sometimes walled, usually with
loose stones, but often have only the natural clay sides. Grass
frequently grows at the margin, and various refuse finds its way
into the water. As numerous examinations have shown, these
ditches are excellent breeding places for *Anopheles febrifer*. We
have found this species abundantly at the grassy margins of
the ditches and occasionally in crannies of the walled sides.
Few other mosquitoes have been found in these ditches. Fre-
quently where the water is very swift in the street ditches small
laterals, often immediately under the houses, furnish favorable
breeding places.

For convenience the "ditch" towns are taken from Table VI
and given in a separate table with additional data (Table VIII).

TABLE VIII.—*"Ditch" or "canal" towns.*

No. in Table VI.	Name.	General surroundings.	Ditches.	Other breeding places.
2	Calamba	Level plain; irrigated rice country.	One main; several laterals; flow comparatively slug-gish. Water from rice paddies.	River, side of town.
9	Lilio	Hilly region near mountains.	Many laterals; clear water, mostly swift, but some quiet places.	Brook, side of town.
10	Magdalena	Plain; coconut-palm region.	Very many laterals; clear water; flow compara-tively slow. Much veg-etation.	Brooks, one on either side of town. Pools near market.
15	Majayjay	Hilly; near moun-tains; elevation, 275 meters.	Many laterals; clear water; flow swift with some quiet places. Some vegetation.	Brook in and near town.

TABLE VIII.—*"Ditch" or "canal" towns*—Continued.

No. in Table VI.	Name.	General surroundings.	Ditches.	Other breeding places.
16	Nagcarlan	Hilly	Many laterals; clear water; flow swift with quieter places. Some vegetation.	Brook near town.
48	Lucban	Mountain; elevation, 425 meters.	Many laterals; clear water; swift. Little vegetation.	Brook in town.
49	Tayabas	Hilly	Many laterals; clear water; mostly swift, but with quiet places. Some vegetation.	Brook near town.
50	Sariaya	Hilly; elevation, 195 meters.	Not many laterals; mostly swift. Some refuse vegetation.	

No. in Table VI.	Anopheles survey.	Malarial survey.			
		Date.	Examined.	Percentage positive.	
				Parasite.	Spleen.
2	*A. rossii* in ditch. *A. rossii* and *A. barbirostris* numerous in river.	Mar. 18, 1914	52	0.0	0.0
		Aug. 6, 1914	116	0.0	0.0
9	*A. febrifer* in crevices of walls of ditches, also elsewhere in ditches and in brook.	Apr. 30, 1914	54	[a]0.0	7.4
		Aug. 19, 1914	50	14.0	
		____do ____	120		1.7
10	*A. febrifer* numerous in ditches, also in brooks. *A. sinensis* (?) in ditch. *A. rossii* in pools.	Mar. 28, 1914	137	19.0	
		July 22, 1914	128	57.8	39.1
		Oct.-Nov., 1914	371	52.6	
		____do ____	292		70.9
15	*A. febrifer* in ditches and numerous in brook.	Mar. 31, 1914	49	8.2	
		Aug. 18, 1914	51	35.3	
		____do ____	118		40.7
16	*A. febrifer* in ditches and in brook	Apr. 29, 1914	53	11.5	9.4
		Aug. 20, 1914	52	28.9	
		____do ____	112		8.9
48	*A. febrifer* in brook. None found in ditches. January 7, 1915.	Dec. 11, 1914	112	3.6	14.4
49	*A. febrifer* in ditches	Dec. 10, 1914	110	29.1	32.7
50	*A. febrifer* in ditch in alley	Jan. 8, 1915	29	4.4	51.7

[a] Slides not in best condition for examination.

The parasite index of all these towns is relatively high with the exception of Calamba, Lucban, and Sariaya. Calamba affords a case of especial interest. One long ditch runs through the main street of the town, and a few laterals extend from it. Quiet places suitable for the breeding of *Anopheles febrifer* are plentiful, but the water in the ditch comes from rice paddies and is comparatively warm and foul. A long search for larvæ

of anopheles in this ditch yielded only a few specimens of A. *rossii*. The parasite and spleen indexes of Calamba were 0.

Lucban and Sariaya show rather low parasite indexes and somewhat higher spleen rates. Each town has many ditches containing clear flowing water, which in the case of Lucban, especially, are plentiful throughout the streets. Both towns are hilly, and the water runs swiftly in most parts of the ditches, but there are many possible breeding places for anopheles. In the survey made of these towns January 7 and 8, 1915, a few larvæ of A. *febrifer* were found in a ditch in a street of Sariaya, but considerable search failed to disclose any in the ditches of Lucban, although a few were found in a brook flowing through the town. Possibly the season of the year may in part account for the fewness of anopheles and the low parasite rate of these towns. Both towns are in a mountain region and rather high (Sariaya, 195 meters above the sea and Lucban, 425 meters), and the climate is comparatively cool. We were informed at Lucban that the worst fever months there are May, June, and July. We do not have sufficient data to indicate whether mountain towns of moderate elevations have malarial rates much different from towns of lower elevations. Antipolo (Table VI, No. 25), with an elevation of from 180 to 190 meters above the sea, shows a lower rate than would be expected taking into consideration the number of brooks flowing through the town and the number of A. *febrifer* and of A. *maculatus* breeding in them. On the other hand, Majayjay, Laguna, with an altitude of 275 meters shows high parasite and spleen rates.

Repeated examinations at different seasons of the year would probably throw some light on these apparent exceptions. However, taken as a group, the high parasite and spleen indexes of these towns, situated in immediate contact with A. *febrifer* breeding places, points to a decided relationship of this species to the transmission of malaria.

The number and distribution of malaria cases and the number and location of the ditches in the town of Magdalena are given on the map (Plate II). The data of a single survey appear on the map, the examination of late October and early November, 1914 (Table VI, No. 10). The blood specimens were collected and the cases located on the map by Rosa, and the slides were examined by Barber. Children of all ages were taken, many of them in a house-to-house canvass. It is evident that both breeding places and cases are uniformly and abundantly distributed.

Before proceeding to a summary of our data regarding the relation of malaria to topography, it may be well to examine the data on certain severe epidemics which have followed the introduction of large bodies of laborers into malarious regions in the Philippines. Three cases occurring in recent years are given in Table IX.

All of these localities are in breeding places of *Anopheles febrifer*, and the epidemics occurred at seasons when the breeding of any stream species of anopheles was not materially interfered with by freshets or drought. In every case there were species of anopheles other than *A. febrifer* and *A. maculatus* present, but of these only *A. rossii* and *A. barbirostris* occurred in any numbers.

The epidemic of malaria which occurred on the Manila–Baguio turnpike in Nueva Ecija during the construction of a bridge over Baliuag River merits a more detailed description. Mr. A. W. Austin, district engineer, who had charge of the work kindly gave us the following information. The epidemic occurred during the months of November and December, 1912, and of January, February, and March, 1913. Some thousands of apparently healthy men, many of them from nonmalarious regions, were brought into a camp on the banks of the river. Some lived in temporary grass huts on an area of dry gravel in the bed of the stream itself. Malaria was so severe that it was necessary to keep 2,400 men on the payroll in order to keep 800 men at work. In one instance, of 300 men who turned out to work in the morning, only 18 were able to work until noon. The number of deaths is difficult to estimate, since many died after returning to their homes, but probably the number of fatal cases went into the hundreds.

This locality was surveyed by Barber on December 31, 1914. The river at this point flows through a plain mostly covered with grass, but with low trees especially near the river. There are no swamps of any kind near. The stream is small, being more like a brook than a river. The water is clear, and flows with alternate swifter and slower stretches. The bed of the stream is gravelly, and there is much flow in the gravel below the surface. There is some vegetation at the margin of the stream, but the banks are cleaner than in the case of many streams examined by us in the Philippines. In a number of places, however, coarse grass extends from the margin into the water. At one such place at the former site of the camp *A. febrifer* was found in abundance, sometimes 8 or more larvæ would be brought up at one dip of the collecting pan. *Anopheles febrifer* was

TABLE IX.—*Recent epidemics of malaria in the Philippine Islands.*

Locality.	Nature of locality.	Epidemic.	Anopheles survey.	Blood examination.
Baliuag River, Nueva Ecija; bridge construction camp.	Plain, mostly grassy with some trees. River or brook with clear water over a gravelly bed; some vegetation at margin. No swamps near.	Nov. and Dec., 1912; Jan., Feb., and Mar., 1913.	Dec. 31, 1914. *A. febrifer*, *A. rossii*, and *A. barbirostris* in brook by camp site.	
Construction line of railroad company, Tayabas Province; Malicboy-South.	Hilly; mostly wooded; part jungle, part coconut groves. Some swamps, salt and fresh. Brooks of varying size abundant.	1914 up to present time. Cases fewer in hot, dry season (Dr. S. R. Cox). Many cases in Nov., 1914.	Nov., 1914. *A. febrifer* abundant in brooks near railroad construction. *A. rossii* and *A. barbirostris* in various localities.	Nov. 11 and 12, 1914. 103 patients, convalescents, and others examined, 72.8 per cent positive. (Barber, examiner.)
San Jose; Mindoro sugar plantation.	Plain, with brook and large river. Numerous irrigation ditches. No extensive swamp.	1911 and 1912. Near end of dry season and early in wet.	May, 1914. *A.* *febrifer*, *A. maculatus*, *A. rossii*, and *A. barbirostris* in brook, river, and irrigation ditches.	May, 1914. Index of latent malaria cases, 37.3. (See Table I.)

also found at other points near the camp. *Anopheles rossii* occurred in algæ and in pools in the bed of the stream. A few larvæ of *A. barbirostris* were also found (Plate I, fig. 2).

Mr. Austin informed us that a camp 6 kilometers beyond the river, away from any stream, remained malaria free.

This epidemic shows clearly the relation between clear streams and malaria in the Philippine Islands. It is said that the people of this region refuse to settle on this river because of the severe type of malaria which prevails there. The stream is reputed by them to be haunted and is known as "Devil River."

In reviewing the data given in Table VI, little can be deduced with regard to distribution by provinces because no complete survey was made of any province, and because the great variety of topography in each province makes a partial survey inconclusive. However, in the two provinces of Cebu and Laguna the number and variety of localities visited were considerable, and some comparison is possible. Cebu, a long narrow island of coral formation, is comparatively poorly watered. The interior is mountainous, and the large proportion of the population is found along the coast. The towns for the most part are but little above high tide. Permanent brooks and rivers are comparatively few, and there seems to be less tendency here than in other parts of the Archipelago to locate towns immediately on the streams, possibly on account of floods.

In September, 1914, Barber made a malarial survey of the province in company with Dr. A. Villalon, an assistant of Dr. Arlington Pond, district health officer of Cebu. On the east coast various localities from Oslob to Sogod, and on the west coast from Tuburan to Aloguinsan, were visited. Mosquito surveys were made and fever cases examined wherever they could be found. In locating fever cases we had the assistance of the local health officers. In addition, the parasite and spleen indexes were taken of school children in the towns of Bolhoon, Carcar, and Argao. The amount of malaria found was small. But few cases of malaria were found, and nearly all of these could be traced to recent residence in a construction camp in Tayabas Province, Luzon, where malaria is very prevalent and where many laborers recruited from Cebu are employed. Only three cases could be found (these in Pardo barrio near Bolhoon) which could not be traced to this source.

Doctor Pond informed us that in ten years of experience in Cebu he has observed comparatively few cases of malaria indigenous in the province and that he has not seen one case of

malarial fever that could be traced to the city of Cebu. Maj. Roger Brook,[17] of the United States Army, reports on the practical absence of cases of malaria contracted in Cebu by soldiers at Warwick Barracks in Cebu city. On the whole, it may be concluded that the amount of indigenous malaria in the province is comparatively small. In some localities we were informed that fever formerly prevailed in places where the jungle was being cleared. The province as a whole is densely populated and relatively free from jungle.

In our mosquito survey of the province all the common species of anopheles were found; *Anopheles rossii* was plentiful, *A. febrifer* occurred in moderate numbers, and *A. barbirostris* and *A. maculatus* were few. With the exception of localities occupied by *A. rossii* there were few breeding places and the number of anopheles was comparatively small. *Anopheles rossii* was found abundantly in some brackish pools in the city of Cebu. Major Brook reports the finding of *A. rossii* and *A. ludlowii* in Cebu.

Laguna Province is in a volcanic region and is well watered by numerous brooks and rivers which flow the year around. There is a great variety of topography, and large areas, especially in the mountains, are covered with jungle. As shown in the tables, the rice-field regions, some of them irrigated the year around, are comparatively free from malaria, while there is a much larger amount of malaria in the hill towns. The province as a whole contains much more malaria than Cebu.

In general in the Archipelago, regions long cultivated are freer from malaria than newer localities. The sparsely settled Islands of Mindoro and Palawan are notoriously malarious, and much malaria is reported from Mindanao. However, the "ditch" towns, which show such a high degree of endemic malaria, are old towns, and what is at present probably the most highly infected place in the Philippines, the line of construction of the Manila Railroad Company in Tayabas, is in part in an old settled region. However, in this place there has been a large importation of laborers, and the railroad line penetrates some areas either original jungle or once cultivated and now reclaimed by the jungle. A region planted with coconut trees, especially, is likely to be allowed to relapse into jungle along the streams and in areas not occupied by the coconut trees. Often the edges and sometimes other parts of towns are practically jungle (Magdalena, Majayjay, and Bongabon). A considerable proportion

[17] *Milit. Surgeon* (1914), **34,** 201.

of the towns which show a high parasite index are situated in coconut regions. Such regions are by no means always malarious. Much of the coast of Cebu, where the bulk of the population is found, is lined by coconut trees. Few of these towns have allowed the coconut groves to relapse into jungle. Factors which favor the dispersal and shelter of mature anopheles must be considered as well as the presence of breeding places; and given sufficient numbers of the proper mosquito carriers, jungle and other overgrowth favor the development of malaria in a community.

In summarizing the results by type of locality, we find that as a rule low-lying coast towns in which the high tide extends into the streams well among or back of the houses show little or no malaria. Parañaque, Orion, and the swampier portions of Manila are good types of towns of this class. In or near these three localities *A. rossii* was found breeding in abundance, but no case of malaria was found in over 700 children examined. Cases of malaria contracted in Manila are few, if, indeed, they ever occur. We have questioned many physicians of the city, and have examined suspicious fever cases obtained in houses or in clinics and have found but few cases positive for malaria, and those almost always gave a history of residence in some other locality, usually a malarious one. The positive cases found in routine examination at the laboratory of the Philippine General Hospital at Manila nearly always show a history of origin of the disease outside of the city. Large cities usually show a lower rate than rural communities.

Bentley [18] states that even small towns of Bengal, India, although intensely malarious, may present areas in their centers nearly malaria free. With a fixed number of anopheles carriers the rate tends to fall as the population increases. However, the portions of Manila examined are for the most part immediately contiguous to swamps or ponds, and if *A. rossii* were a carrier we would expect at least a small positive spleen or parasite index.

Mangarin in Mindoro and Limay in Bataan Province are exceptions to the rule that low coast towns are malaria free. Mangarin, which shows a parasite index of 39.3, is situated on a flat swampy coast. In the dry season there is no fresh flowing water within a kilometer. From the nature of the country it is probable that fresh streams are found much nearer to the town in the wet season. Palangeran River flows into the sea

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[18] *Ind. Journ. Med. Res.* (1914), 4, suppl. 78.

at from 1 to 1.5 kilometers from the town. At the time of our survey the larvæ of *A. febrifer* were more numerous in this river than in any other stream, except one, surveyed in the Philippines. Doctor Daywalt informs us that in the wet season there is a spring in the town itself at the margin of which larvæ of *A. febrifer* were found. He sent us some specimens of mature anopheles caught in houses in the town during the rainy season and among these was one *A. febrifer*. So it is evident that this species either breeds in or near the town or is able to reach it by flight from a distance. Mangarin is situated in a highly malarious region, and there is more or less intercommunication with the inhabitants of the San Jose Estate and other places where malaria is, or has been, very prevalent.

Limay, Bataan Province, shows a parasite index of only 6.8. It is situated on the coast near the foot of the mountains, and a lumber mill is situated in it and connected by a railway with the mountains at the foot of which the town lies. Shortly before this region was surveyed for mosquitoes, there had been a freshet, so that few larvæ of any kind were found, but a few larvæ, apparently *A. febrifer,* were found in a brook at the edge of the town.

Of the coast towns situated well above high tide, Puerto Princesa, Palawan Island, shows a parasite rate of 28.6. Numerous larvæ of *A. febrifer* and *A. rossii* were found in a small stream at the edge of this town. Like Mangarin, Puerto Princesa is in more or less communication with a highly malarious region.

The results of this work tend to confirm the preliminary conclusion of Walker and Barber that the chief carrier of the Philippine Islands is *Anopheles febrifer*. The mosquito survey has shown the wide distribution of this species, the abundance of its breeding places, and the large numbers of larvæ often found in them. Further it has been shown that this species is house-seeking and readily bites human beings. Its distance of flight is at least 170 paces, and the number found in houses in some localities was large in comparison with the number of larvæ found in the neighboring breeding places.

The distribution of malaria agrees, in general, with the distribution of *A. febrifer*. In every malarious town in which it was possible to make an adequate anopheles survey, either larvæ of *A. febrifer* were found in or near the locality or adults were caught in the houses. The high rate of malaria occurring in "ditch" towns where *A. febrifer* breeds in close proximity to houses tends to strengthen the evidence against this species.

It is true that *A. febrifer* was found breeding near towns with little or no indigenous malaria, but the breeding places were in most cases more or less remote from the majority of the houses or comparatively few larvæ were found in them. The lack of gamete carriers might in some cases account for the lack of malaria in these localities. However, none of the localities surveyed were found nonmalarious where *A. febrifer* bred abundantly near houses and had unobstructed flight to them. Possible exceptions are Bolhoon, Cebu, and some more or less recently established barrios on large plantations. Many factors are concerned in the transmission of malaria, and as investigations in many localities of other countries have shown, the juxtaposition of mosquito malaria carriers and of population is not necessarily accompanied by malaria.

The results of our study of *Anopheles rossii* as a whole tend to confirm the conclusions of investigators in India that this species is responsible for little if any transmission of malaria. There is probably no locality in the Philippines where this species does not breed at some time during the year and in most places very plentifully. If it were a carrier, we would expect a correspondingly wide distribution of malaria, yet our survey shows that there are many localities where *A. rossii* breeds at almost all times of the year in large numbers and close to houses, yet these localities show an index of 0. This is true of towns like Manila, Parañaque, and Cebu city and other coast towns of Cebu where *A. rossii* breeds abundantly in salt or brackish water, and in towns like Calamba in Laguna Province and Orion in Bataan Province, surrounded by rice paddies, which afford fresh-water breeding places the year around.

There also seems to be little correlation between the breeding places of *A. barbirostris* and *A. sinensis* and the presence of indigenous malaria. The occurrence of both in irrigated rice paddies where the malarial index has been found to be low indicates that they are not important carriers. *Anopheles barbirostris* and *A. sinensis* do not seem to be by preference house-seeking species.

Anopheles maculatus probably transmits malaria in certain localities and at certain seasons in the Philippines. In December, 1913, this species occurred in considerable numbers in a brook at Canlubang. On the banks of this brook a barrio is located in which considerable malaria occurred at that time and earlier in the season, and it is possible that *A. maculatus* was a carrier. At Camp Stotsenberg, Pampanga Province, this species

occurred in the immediate neighborhood of a malarious barrio. In both localities *A. febrifer* was also found. However, *A. maculatus* is relatively scarce, and probably plays a far less part in the transmission of malaria than *A. febrifer.*

Watson [19] believes that *A. maculatus* (= *A. wilmori*) is the chief carrier in certain hill regions of the Federated Malay States. He reports this species from a Dutch island off Singapore, where malaria is intense, and from Hongkong. In any case, antimalarial measures directed against *A. febrifer* would be equally efficacious against *A. maculatus*, which has similar habits.

In general, the highest indexes of malaria have been found in well-watered, but not necessarily swampy regions; and small clear streams, especially where in close proximity to houses, offer more danger than swamps, lakes, or wholly stagnant water.

SEASONAL INDEX OF MALARIA

From information obtained locally in malarious towns regarding the worst "fever" months, it would appear that March, April, and May, in the hot dry season, are most free from malaria, but the evidence is not without contradictions. For the rest of the year the information obtained varied widely in different localities, but the rainy season and the cooler dry months were more often mentioned as malarious. Where the number of latent cases is large, we would expect a proportion of these cases to become acute at times when there is the most exposure to weather—the rainy season and the cool season, when the nights are often decidedly chilly and thinly clad people suffer from cold. As shown in Table VI, towns examined twice give a percentage of positives obtained during the hot dry season lower in nearly every case than that obtained between July and December 1. In some cases the slides obtained in the hot months were in poorer condition for examination, but making allowance for this there is still a lower percentage for the hot dry season.

A very important matter in the epidemiology of malaria is the time of the year when most infections occur. This is a matter difficult to determine from statistics obtained from the indigenous population of a malarious district where a large percentage of the people harbor parasites the year around. Some information may be obtained from isolated epidemics

[19] *Trans. Soc. Trop. Med. & Hyg.* (1913), 59.

where a comparatively malaria-free population has been introduced into a malarious district. Dr. G. W. Daywalt informed us that a group of about 400 laborers came to the San Jose Estate in February and March, 1911. Within two weeks 90 per cent of them were attacked by malaria of a pernicious type, and many of them died. This was in the dry season. Generally, according to Doctor Daywalt, the severest epidemics on the San Jose Estate have begun in May, a hot month with frequent afternoon showers.

On the line of construction of the Manila railroad where malaria was very prevalent at least up to November, 1914, the fewest cases appeared in the hot dry season, according to Dr. S. R. Cox, who until recently was resident physician there. Barber surveyed this region during the dry season and again in November. Evidently there is much more flow of water in the brooks, which are plentiful there, after the rainy season, and some brooks go dry, or nearly so, in the dry season. Laborers were continually being introduced into this region from Cebu and other relatively nonmalarious regions.

The severe epidemic at the bridge-construction camp at Baliuag River, in Nueva Ecija Province, occurred in the dry season—that is, November, December, January, February, and March. The epidemic on the line of construction of the Manila water works in 1906 occurred in February.

The severe outbreaks mentioned above have occurred near the habitat of stream-breeding mosquitoes and at times when their breeding was not interfered with by freshets or drought. In the "ditch" towns (Table VIII) the supply of water is often so regulated that breeding is less interfered with by heavy rains. We found a very high rate of infection in Magdalena, Laguna Province, in July, and the people in this town state that the fever is worse during the rainy season.

We believe that the results of our work give a more optimistic outlook for successful antimalarial work in the Philippines. Large parts of the more populous portions of the Islands contain little or no indigenous malaria, and the people residing in them are little exposed to infection. It is probable that the amount of morbidity and mortality from malaria in the Philippines has been overestimated. The following data were kindly furnished us by Dr. Victor G. Heiser, Director of the Bureau of Health of the Philippine Islands:

Annual death rates and morbidity rates from malaria in the Philippine Islands, 1909 to 1913, inclusive.

Year.	Deaths from malaria.	Total deaths.	Average.	Cases reported.	Population.	Average.
1909	a 25,751	179,355	14.35	a 16,516	6,331,695	0.26
1910	26,359	191,576	13.75	10,225	6,956,979	0.14
1911	28,181	188,412	14.95	19,363	7,007,081	0.27
1912	27,229	184,639	14.74	11,555	6,857,892	0.16
1913	17,619	147,544	11.94	20,378	6,770,736	0.30

a In the first four years the deaths from malaria exceeded the cases reported because of incomplete returns from the provinces.

Dr. Arlington Pond, district health officer for Cebu Province, gave us the following information by letter:

In this year (1912) there were over 4,000 deaths reported from malarial fever (Province of Cebu). The following year I employed eight doctors and divided the province up into districts. As the result of this the number of cases of so-called malaria dropped to 400 instead of the 4,000 of the previous year.

From results obtained during our survey of the province in 1914, it would seem that even this figure is far above the actual rate. It is probable that if facilities were available for more accurate diagnosis in all provinces a far lower morbidity and mortality rate for malaria would be reported.

If, as our results indicate, the greater part of the transmission of malaria is due to a species of mosquito of rather limited habitat, the outlook is encouraging for eradicating or greatly reducing the mosquito carriers of malaria in many malarious localities. An anopheles-mosquito survey, and wherever practicable a malarial survey, should be made of every locality where antimalarial work is contemplated. Wherever children in schools are available for blood and spleen examination, the examination of 50 persons can be completed by a trained worker within three days at the most. The data thus obtained are of the greatest importance as a basis for antimalarial work. Clinical evidence alone is much less satisfactory unless obtained by a trained diagnostician who has resided some time in a locality.

With the scientific data at hand the next step is to choose the point of attack best suited to the locality and the resources available. In the "ditch" towns, for example (Table VIII), the attack can be most advantageously made against the larvæ in the ditches. The most radical measure would be simply to abolish the ditches by cutting off the main canal and to rely on water

supply from other sources. Water equally good, even if a little less conveniently at hand, is found in most of these towns within a hundred meters or less. Again, the open sewers and other ditches under the houses could be cut off and only two or three ditches in the main streets retained. These might be cemented at little cost, or, if this much expense is impossible, they could be kept clean and their channels so arranged that the water could have swift and unobstructed passage and the larvæ could be kept down by the use of larvicides. Near many towns not of the ditched type the breeding places of *Anopheles febrifer* are very limited. In Puerto Princesa, for example, we could find but one such breeding place anywhere near the town. One or two hours' work by one person would suffice to destroy practically every anopheles larva in that place.

In localities where one or more streams flow through or near the houses, the destruction of the larvæ is more difficult, but still quite practicable. As a rule, the breeding places of anopheles do not occupy the whole margin of the brooks, but occur here and there where vegetation offers some protection. Cleaning out these breeding places would accomplish much and in many streams we have surveyed would require comparatively little time and expense.

Permanent good may be accomplished by clearing the stream bed so as to make it narrower and swifter and to do away with some of the pools and quieter water where the larvæ breed. Work of this sort has been done successfully at the Calamba Sugar Estate.

Watson [20] brought about a great diminution of the malaria rate in certain estates in the Federated Malay States by subsoil drainage of the clear streams where *Anopheles maculatus* breeds. This was done only for certain sanitary areas, and on account of the expense such permanent improvements would be difficult to carry out in most localities in the Philippines. Larvicides are cheaper and at the present offer a more practical method for mosquito destruction in these Islands.

Since the larvæ of *Anopheles febrifer* and of other stream breeders have the habit of hiding in crevices in the bank and under vegetation, it is difficult to apply a larvicide adequately. However, this characteristic renders it unnecessary to treat any part of the stream other than the bank. Barber has made some experiments in the destruction of the larvæ of *A. febrifer,* the results of which are given in Table X.

[20] Loc. cit.

TABLE X.—*Experiments with mosquito larvicides.*

Experiment No.[a]	Date.	Locality.	Nature of breeding place.	Approximate width of stream.	Preliminary survey.		
					Station No.	Approximate dips.	Larvæ.
	1914			*Meters.*			
1	Apr. 14	Canlubang, Laguna	Rocky brook with much vegetation and jungle.	0.5-5	14	14	128
2	Apr. 15	Santa Rosa, Laguna	Swift clear brook; comparatively little vegetation.	2 -6	11	11	113
3	May 16	San Jose, Mindoro	Small brook; moderate amount of vegetation.	0.5-3	10	10	65
4	July 31	Magdalena, Laguna	Narrow ditch; much grass. Water cut off half an hour before oiling.	0.3	--------	26	113

Larvicide.		Approximate distance worked.	Approximate time.	Interval between spraying and reëxamination.	Survey after use of larvicide.		Average per dip.		Ratio per dip before and after.
Kind.	Amount.				Dips.	Larvæ.	Before.	After.	
	Liters.	*Meters.*	*h. m.*	*Hrs.*					
Crude petroleum	2	1,000	3 0	20	200+	7	9.1	0.035	100:0.4—
Larvicide, Darling.	7	400	30	24	137	38	10.0	0.280	100:2.8
Crude oil	8	60	15	48	--------	8	6.5	(c)	[d]100:1
				5	81	1	4.3	0.012	100:0.3
Kerosene	4.5	370	20	16	55	0	4.3	0.00	100:0.0
				(b)	many	0	4.3	0.00	100:0.0

[a] The depth of Nos. 3 and 4 was only from 3 to 5 centimeters except where very narrow. No. 1 averaged from 10 to 20 centimeters where the water was swift, and No. 2 had two or three times the flow of No. 1. In Nos. 1, 2, and 3 brooms were used to work the larvicide into the bank. In No. 4 kerosene was put on the water and simply splashed on the sides of the narrow ditch. Nos. 1 and 4, larvicide applied by one person. Nos. 2 and 3, larvicide applied by two persons, each taking one side of the bank.

[b] 19 days.

[c] After treatment larvæ found only two or three times along the whole distance and in very small numbers. Exact data not recorded.

[d] Estimated.

Preliminary experiments showed that the larvicide when merely poured on a stream and allowed to float down was ineffective, especially where the current was swift. So the larvicide was distributed along the margins and worked in mechanically. We found one of the best methods of distributing the oil was by means of a can provided with a long spout, and for working it into the breeding places we used, in most experiments, Filipino brooms made of stiff twigs. This method

is not so time-consuming as it appears, since, in experiment 1, one of us "cleaned" both sides of a very much obstructed brook for a distance of nearly 1 kilometer in three hours with no help except that of assistants to carry a supply of crude oil. The brook was much obstructed by trees and jungle and was one of the worst in this respect of any we have visited in the Philippines.

In order to control the results of the larvicide in our experiments, the whole distance to be treated was first surveyed. At certain stations dips were made at the margins with the collecting dish, the larvæ obtained were counted and replaced in the water, and the results were entered in a note book. The stations were marked, usually with numbered stakes made conspicuous with strips of red cloth. At a known interval of time after the application of the larvicide, dips were again made at the stations and at points between them, and the larvæ counted. The dips were made in places where by long experience in collecting we knew that larvæ were likely to be found. The streams in all experiments contained clear flowing water, and there were few larvæ other than of *Anopheles febrifer* present. There had been no preliminary clearing away of breeding places. In experiment 4 the water had been shut off from the ditch half an hour before the application of the kerosene, and was not turned on again for some hours. As a result, the quantity and the movement of the water were diminished, and the larvicide was made more effective. After the application of the larvicide the stream was searched for larvæ before the water was turned on, and was searched a second time some hours after the water had been allowed to flow into it.

The percentage of larvæ destroyed can be estimated only approximately, but it is apparent from the data given in the table that from 95 to 100 per cent of the larvæ of *Anopheles febrifer* may be destroyed by one application of any good larvicide where it is well worked into the breeding places. Where the breeding places are limited to one or two small streams in or near a town, as is frequently the case in the Philippines, one trained person could keep down the larvæ for a distance of at least several hundred meters above and below the town.

Our data also show that the time and expense required are not great. In our experiments we "scrubbed" the entire margins of both banks, except at certain points which were left as controls. If we had confined the treatment to known breeding places, much time and larvicide might have been saved. However, where the larvicide is applied by a person unfamiliar with

the breeding places, it would be inadvisable to skip any part of the bank.

A practical application of our method of larva destruction has been made by Dr. I. S. Diller, of the Calamba Sugar Estate. The brook treated by us in experiment 1 flows near a large barrio of the estate. This brook has been systematically treated opposite and some distance below the barrio by an assistant whom we trained. Doctor Diller reports a diminution both in the number of mosquitoes and in the cases of malaria in the barrio.

We have not observed much tendency in these larvæ to follow the current down the stream, except when there is abundant floating débris for them to cling to. In experiment 4 the treated ditch was found practically free of larvæ nineteen days after the ditch had been opened to the main ditch above, where larvæ were plentiful. It is probable that if a portion of a stream is thoroughly cleared it will remain so until a fresh supply of larvæ have had time to develop.

As stated above, we found that larvicide merely poured on the brook was comparatively ineffective. One experiment conducted on the same brook as that described in experiment 1, Table X, may be described in detail. Twelve dips at 12 stations gave, before treatment, 150 larvæ. The distance was approximately 100 meters. Then about 3 liters of crude oil were poured on the stream at the upper station. Twenty-four hours later the brook was again examined. Twelve dips at the stations gave 36 larvæ, and 78 additional dips above and below the stations gave 95 larvæ, a total of 131 larvæ. The average numbers obtained per dip were 12.5 larvæ before treatment, 1.5 larvæ after treatment. The destruction of the larvæ was very marked where the current was comparatively slow and the margin of the bank little protected by vegetation, but it was very little where the current was swift and the bank protected by overhanging roots and ferns. In experiment 1, Table X, certain stations were left untreated as controls. These stations showed decidedly less diminution of the larvæ than did the places where the larvicide was worked in.

In Magdalena, at the same time that experiment 4 was made, a ditch on another street was partially dammed, and a quantity of petroleum was placed immediately below the dam and at other points below. Then the water was liberated so that the larvicide could be carried down by the rush of the stream. There was some destruction of larvæ, but the results were not wholly satisfactory. A second experiment of the same sort also gave indifferent results. It seems that it is necessary to work the

larvicide in mechanically in order to destroy the larvæ of these stream-breeding species, especially where much vegetation is present. In the walled ditches passing under houses or into other inaccessible places a simpler treatment might be devised.

As stated above, *Anopheles febrifer* and other stream breeders avoid rice paddies and water in ditches which has previously stood on large paddies. Moreover towns in rice-paddy regions where there are few brooks or other streams are comparatively malaria free. In Calamba, Laguna Province, a ditch from a rice paddy flows through the main street of the town, and laterals extend to other streets. We could find no anopheles other than *A. rossii* in this ditch, and the malarial index of the children of the town is 0.

Watson[21] remarks on the fewness of stream breeders and the comparative absence of malaria in certain rice-paddy areas in the Federated Malay States while malaria is plentiful in the neighboring hill regions. He believes that irrigation as well as drainage may in some localities be an efficient antimalarial measure.

Kendrick[22] finds that in the irrigated rice regions of central India two factors must coexist in order to bring about a high rate of endemic malaria: namely, breeding places of anopheles-mosquito carriers and shade in the form of trees, shrubs, long grass, or other jungle.

Not all the species of malaria-bearing anopheles of central India are found in the Philippines. Climate and other conditions differ so that we do not expect the distribution of malaria here to correspond closely with that of India, but it is worthy of note that in the Philippines as well as in central India open irrigated rice regions may be nearly malaria free. Generally malarious localities in the Philippines are on streams which come more or less directly from wild land or land uncultivated for many years. In some parts of the Philippines the further development of rice culture may result in the diminution of malaria. However, water in irrigation ditches, if it has not previously stood on extensive rice paddies, affords good breeding places for *Anopheles febrifer*.

While drainage has undoubtedly played a large part in the diminution of malaria in various regions—for instance, the middle west of the United States—it seems that extensive cultivation of the soil might also have been an important factor.

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[21] Loc. cit.
[22] *Ind. Journ. Med. Res.* (1914), 4, suppl. 64.

Not only the diminution of water but the rendering of it unfit for malaria-carrying mosquitoes is to be considered. Whether a change in water may affect the susceptibility of the mosquito to malaria without impairing its breeding is a question which cannot be solved with the data now at hand.

Among the natural enemies of stream-breeding mosquito larvæ we have most often encountered a species of *Ranatra*.[23] We have observed the insects and larvæ of a species of the family Dytiscidæ[23] feeding on mosquito larvæ in streams. In a shallow pool containing *Anopheles rossii* we have observed flies catching larvæ. The flies rested on twigs and on the surface-tension layer of the water and seized the larvæ from above when the latter came to the surface to breathe. In a brackish pool in Palawan Province tadpoles were observed catching the larvæ of a species of *Culex*. These tadpoles had developed legs, but were still gill-breathing. A specimen was put into formalin and later dissected in the laboratory where mosquito larvæ were found in the upper part of its digestive tract.

Where larvæ are protected by algæ, grass roots, stones, or floating débris, we have observed that they breed in large numbers in spite of the immediate presence of their enemies. This has been the observation of most workers in various countries. The rapid destruction of larvæ in breeding jars by natural enemies is not a reliable index to the behavior of these enemies in the natural state. It is apparent that natural enemies cannot alone be depended on effectively to keep down anopheles. The introduction of larvæ-destroying fishes or other mosquito enemies should be practiced wherever possible. They are most effective in ponds, tanks, basins, or other still waters, but we would not hope for much success from them in streams where larvæ are well protected.

The destruction of adult mosquitoes in and about houses, especially those which have taken blood, has been one of the means successfully used in antimalarial work in Panama. As stated in the description of our work in Mindoro, Doctor Daywalt, resident physician of the San Jose Estate, keeps a squad of mosquito catchers employed, and he attributes to this work no small part of the reduction of malaria on that estate. The success of this measure must depend to a great degree on assistants who will carry on the work persistently and throughout the year.

[23] Identified by C. S. Banks.

In the Philippines it is doubtful if enough of such assistants could be trained and kept at work in the various malarial towns. The hiding places of adult mosquitoes are not necessarily confined to buildings. We found 2 adult females of *Anopheles febrifer* among roots and in a crab hole in Mindoro. The average Filipino room in the Philippines is open to the high thatched roof, and the space under the house also offers lurking places not easily accessible. The houses on the San Jose Estate are for the most part provided with ceiling, and the under part of the houses are so ventilated that they are not favorable as hiding places for mosquitoes. As a result, mosquito catching on this estate is probably easier than would be the case in the average Philippine barrio. This kind of antimalarial work will probably be most likely to succeed on plantations or other localities where the work can be kept under close supervision.

Educational work is certainly worth while, if only to make the people more receptive to antimalarial measures conducted by the authorities. We have several times talked to pupils or teachers in malarial towns on malaria transmission, and shown them specimens of anopheles breeding in the vicinity.

The clearing of the jungle, whether woods or high cogon grass, has undoubtedly been a factor in the reduction of malaria in some parts of the Archipelago. But, as stated above, the jungle, especially in some coconut regions, has been imperfectly cleared or allowed to grow again, and some of the most malarial towns in the Islands have been settled for a century or more (Magdalena and Majayjay, Laguna Province, and Bongabon, Nueva Ecija Province).

The penetration of new territory in the Philippines by troops or by men employed in construction works or in the development of mines, plantations, or lumber industries has frequently been followed by severe outbreaks of malaria. We believe that much of this malaria could be prevented by comparatively inexpensive antimalarial measures undertaken early and based on an adequate preliminary anopheles survey and where practicable a malaria survey of the indigenous population. In the case of an establishment in a hilly region where breeding places are comparatively limited the destruction of larvæ in the neighborhood of camps would be especially feasible. Very little expenditure of time and money would have sufficed to prevent the outbreak on Baliuag River in Nueva Ecija (Table IX). A small construction camp in Cebu is located immediately over a small brook offering excellent breeding places for anopheles, but we were

unable to find more than one or two larvæ after considerable search. We found that the foreman of the camp had been using moderate amounts of crude carbolic acid in this stream. We could find no malaria in the camp, and there were few mosquitoes about.

A large proportion of laborers recruited from the large centers of population in the Philippines are undoubtedly little infected at the start, and as experience has shown they offer little or no resistance to the disease. When brought into construction camps they have suffered severely with malaria. Once well infected it is difficult to eradicate the epidemic in the camp, and as such populations are rarely permanent, gamete carriers are spread throughout the country. The supervision of such camps and the prevention of malaria in them becomes a matter of general as well as of local importance.

We have had no opportunity of judging the success of quinine prophylaxis in any locality in the Philippines except at the Iwahig penal colony in Palawan, where 5 grains of quinine per day are given to each colonist. The amount of acute and latent malaria in this colony and the results of the mosquito survey there are given in Table IV and the accompanying text. Since anopheles mosquitoes are abundant there and the region is very malarious, it is probable that the prevalence of malaria in the colony is materially reduced by the prophylaxis, but as the data show it is by no means wholly prevented. Quinine prophylaxis is generally considered advisable only as a temporary measure or in an intensely malarious region where more permanent antimalarial means are impracticable.

Measures have already been taken by the Bureau of Health by which quinine is made available to people in many localities at comparatively little cost. Such measures are not only of the greatest value in curing the sick, but the number of gamete carriers is undoubtedly reduced. It is difficult, however, to get people to follow a quinine treatment persistently enough to get rid of latent malaria and consequently of the gametes of the parasite, and many probably do not take quinine at all. Therefore, in order to get permanent results, the distribution of quinine should be supplemented by measures for the prevention of the transmission of the disease.

Bed nets, efficacious where intelligently used, are not to be relied on for an ignorant population, as shown by the results of the mosquito survey at Iwahig where many anopheles were found inside of badly adjusted nets. Many people in the Islands

are averse to the use of bed nets. In a temporary hospital in a very malarious region in Luzon, which we visited before daylight in order to observe the behavior of mosquitoes, we found but few of the nets in use, although nearly all beds were provided with them.

In summary, we believe that the destruction of larvæ by larvicides and where practicable by the abolition of breeding and lurking places offers more encouragement than any other antimalarial measures in the Philippines. These measures should be supplemented by others as conditions advise. Where resources are adequate, all breeding places of all mosquitoes should receive attention, but in case means are limited, the stream breeders, *Anopheles febrifer* and *A. maculatus,* should be eradicated or much reduced, and the streams should be freed from them for as great a distance as possible from towns or camps. The destruction of these species is made easier by the restricted nature of their breeding places, and the cleaning of a very jungly stream has been shown to be a practical possibility.

Filariæ were found only once during this work. No special search was made for them, but species occurring in the blood during the day must be very uncommon among Filipino children, else they would have been oftener observed in the course of examination of thousands of thick smears. In the one positive case, an adult Japanese at Canlubang, filariæ were found in blood specimens taken at midday on several successive days, but were fewer than in specimens taken early in the morning.

GENERAL SUMMARY

1. The commonest species of *Anopheles* in the portion of the Philippines covered by our survey are *A. rossii, A. febrifer, A. barbirostris, A. maculatus,* and *A. sinensis.* It is probable that these are the commonest species of anopheles over the whole Archipelago.

2. The results of this work and that of the work of Walker and Barber indicate that *Anopheles febrifer* and, to a less extent, *A. maculatus* are the chief transmitters of malaria in the Philippines.

3. *Anopheles febrifer* is a stream breeder widely distributed and often occurring abundantly in the breeding places. It seeks houses and readily bites human beings.

4. The distribution of malaria in the Philippines, as indicated by nearly 7,000 examinations of spleen or blood of school children, is by no means universal but is most abundant in

such regions as afford breeding places for *A. febrifer* and *A. maculatus.*

5. Antimalarial measures should be based upon a thorough anopheles and malaria survey, and those measures should be employed which will best meet the conditions. The best single measure is the destruction of larvæ of malaria carriers, and in this work the breeding places of the stream breeders should receive first attention.

6. Our own experience and that of others in the destruction of stream breeders by means of larvicides leads us to believe that this measure is a practical one in the Philippines and that it is within the means of many malarious communities in the Archipelago to reduce the amount of malaria by this measure.

ILLUSTRATIONS

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Fig. 1. A stream flowing through Bongabon, Nueva Ecija, a town with high malarial index.

Fig. 2. Baliuag River, Nueva Ecija. Larvæ of Anopheles febrifer were found in the grass at the margin of the stream at the right.

PLATE I.

MAGDALENA, LAGUNA PROVINCE, P. I.
FROM A MALARIAL SURVEY MADE IN LATE
OCTOBER AND EARLY NOVEMBER, 1914

The symbols represent cases of malaria in children
15 years of age or less found in the houses

Subtertian
Tertian
Quartan
Negative
Child not examined
Ditches containing
    clear flowing water

10 0   50   100
METERS

to Majayjay

CALLE WASHINGTON

CALLE M. DEL PILAR

CALLE J. TOLENTINO

Posage de Luna

CALLE AGUINALDO

CALLE BURGOS

CALLE GOMEZ

CALLE ZAMORA

Old Market

Municipal Building

CALLE CAILLES

CALLE HERRERA

New Market

Railroad

R.R. Station

CALLE J. BERNARDO

Central School

Church

MAGDALENA, LAGUNA PROVINCE, P.I.
FROM A MALARIAL SURVEY MADE IN LATE
OCTOBER AND EARLY NOVEMBER, 1914

The symbols represent cases of malaria in children
15 years of age or less found in the houses

Subtertian
Tertian
Quartan
Negative
Child not examined
Ditches containing
clear flowing water

10 0   50   100
METERS

CALLE TAFT

to Mojayjay

CALLE L. JAENA

CALLE ARELLANO

Hipunon

PLATE II.   MAGDALENA, LAGUNA PROVINCE, LUZON, SHOWING THE RESULTS OF A MALARIAL SURVEY OF THE TOWN.

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PATHOLOGIC ANATOMY OF BUBONIC PLAGUE [1]

By B. C. CROWELL

(*From the Department of Pathology and Bacteriology, University of
the Philippines, and the Biological Laboratory, Bureau
of Science, Manila, P. I.*)

FIVE COLORED PLATES

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INTRODUCTION

The present study is based on 75 cases of bubonic plague
that have been autopsied in Manila during the period between
June, 1912, and June, 1914. Other extensive studies on the

[1] Received for publication May 7, 1915.
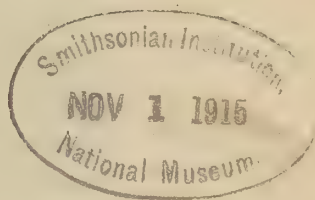
same subject have appeared from time to time, and these will be referred to in detail in this study, but confusion still reigns concerning some phases of the pathologic anatomy of bubonic plague.

This study was undertaken primarily for the purpose of correlating the plague material on hand. Especial attention has been given to accuracy of observation of isolated facts, to the correlation of these facts with allied facts as exemplified in other acute infections, and with the principles that have been deduced therefrom to form the laws of general pathology. A perusal of the literature of the pathologic anatomy of bubonic plague and a study of our own cases seemed to make a study from this general standpoint especially desirable in this disease.

The abundant material has naturally furnished some valuable data relative to the frequency of the occurrence of various lesions in the disease. In the course of the work it has been possible also to draw some definite conclusions concerning the association of some of the lesions in bubonic plague.

An attempt has been made to simplify the classification of cases of plague by recognizing only the primary bubonic and primary pneumonic forms and placing all other previously recognized types under these two forms. This is very simple, save in that ill-defined class of cases spoken of as "septicæmic plague." From a review of the literature on the subject and from a study of our own cases reasons have been deduced for calling these cases "bubonic plague with early septicæmia," in the belief that this phrase more accurately describes them. The further classification of plague cases that is suggested appears to satisfy both anatomic and clinical requirements, and is based on the prominent lesions other than the bubo.

A previous study of a series of primary pneumonic-plague cases excited interest in the relation between the incidence of cervical buboes, tonsillar and pharyngeal lesions, and pulmonary lesions. This relation has particularly engaged the attention in the present study of bubonic cases, and interesting facts have been elicited. In brief, it has been found that there is no constant relation between the lesions of the tonsils, cervical lymphatic glands, and the lungs. Specific pulmonary and tonsillar lesions may occur together or separately in cases with primary buboes in distant parts. On the other hand, primary tonsillar lesions or primary cervical buboes sometimes are and sometimes are not followed by specific pulmonary lesions. The classification of pulmonary lesions adopted follows very closely that suggested by the Austrian Commission. The term "primary

tonsillar bubo" has been introduced to designate the lesion occurring in the tonsil when that organ forms the portal of entrance of the bacillus to the organism, and it appears that such "tonsillar buboes" are associated with primary buboes of the second order in the prevertebral glands, whereas primary buboes of the parotid or submaxillary lymphatic glands are not associated with tonsillar lesions.

It appears of importance to emphasize the occurrence of pharyngeal lesions in cases without pulmonary involvement, since these, as well as the pulmonary cases, may have infective sputum. The more thorough the study of bubonic plague, the greater appears the number of methods of possible direct and indirect transmission of the disease. In regard to the other viscera the particular features brought out in the present work are the relative infrequency of specific focal plague lesions except in the skin and the relative frequency of that lesion of the kidneys to which Herzog especially directed attention: namely, fibrin thrombosis of the glomerular capillaries, which was present in at least 41 per cent of my cases. Two notable cases of plague meningitis have also been encountered in this series.

SOURCE AND CHARACTER OF MATERIAL

Bubonic plague appeared in Manila in June, 1912, 89 cases occurring up to June 13, 1914. In rats the disease is known to have been present since August 31, 1912, and 49 plague-infected rats were found up to June 13, 1914. Seventy-five of the human cases proved fatal, and post-mortem examination of all of these cases was made.

All fatal cases were autopsied at periods varying from a short time to two or three days after death; two of them were performed after extensive putrefactive changes had taken place, the bodies having been previously buried. In all except these two cases the bodies were in a good state of preservation. The anatomic diagnosis was always confirmed by smears, cultures, agglutination of cultures, or by guinea-pig inoculations of portions of tissue removed from various parts of the body. In all of the earlier cases the bacteriological investigation included all four of the above procedures, as was also true in the later cases in which there was any possibility of doubt as to diagnosis. The tissues selected for routine bacteriological examination were from the buboes and spleen.

Mortality.—Up to June 13, 1914, 89 cases occurred in Manila, 75 of which were fatal. The mortality was, therefore, 84.27 per cent.

Race.—Fifty-eight of the cases were Filipinos, 16 were Chinese, and 1 was an American.

Sex.—Sixty-two of the fatal cases were in males and 13 in females.

Age.—The age of the patients ranged from 5 months to 56 years. Table I shows the age incidence.

TABLE I.—*Age incidence of fatal plague cases.*

Age.	Cases.	Age.	Cases.
Under 1 year	1	21 to 25 years	10
1 to 5 years	2	26 to 30 years	12
6 to 10 years	3	31 to 40 years	14
11 to 15 years	9	41 to 50 years	7
16 to 20 years	15	51 years and over	2

Duration of illness.—The average duration of illness was five days. The occurrence of 6 cases with illness lasting from ten to fifteen days makes the general average higher than it otherwise would have been. In more than half the cases the duration of illness was five days or less. Table II shows the duration of illness.

TABLE II.—*Duration of illness in fatal plague cases.*

Duration of illness.	Cases.	Duration of illness.	Cases.
Days.		*Days.*	
1	5	9	1
2	6	10	3
3	15	11	0
4	12	12	0
5	9	13	0
6	6	14	1
7	9	15	2
8	2	unknown	4

The epidemic which furnished the material for this study was entirely of cases of the bubonic type, and it is with that type alone that this paper will deal. The epidemiologic and bacteriologic aspects of this same epidemic have been the subjects of papers by Heiser[2] and by Schöbl[3]. They have shown a direct relation between the incidence of the disease in rats and human beings in this epidemic. The origin of the epidemic is unexplained, the first recognized case occurring in a native who had not been out of Manila. Heiser ascribes it to the importa-

[2] *This Journal, Sec. B* (1913), **8**, 109.
[3] Ibid. (1913), **8**, 409.

tion of rats in cargo from China, since China at the time had infected ports from which vessels were constantly arriving.

CLASSIFICATION OF PLAGUE CASES

BUBONIC AND PNEUMONIC PLAGUE

It is known that *Bacillus pestis* may produce two types of disease which differ in their epidemiologic, symptomatologic, and pathologic aspects. This difference in the type of disease caused by the same microörganism is dependent on the portal of entry into the host and on the condition of the atmosphere in regard to temperature and humidity.[4] When infection occurs through the skin or exposed mucous membranes, the bubonic type of the disease occurs and is manifested usually by enlargement of the superficial lymphatic glands draining the area inoculated, by fever and prostration, sometimes by extensive cutaneous symptoms, sometimes by marked pulmonary symptoms, and frequently by marked cerebral symptoms. When the infection takes place through the respiratory tract, the primary pneumonic type of the disease occurs with symptoms chiefly referable to the lungs. The bubonic type is said to be transmitted chiefly from infected rats to the human being through the agency of the rat flea (*Loemopsylla cheopis*). Attention has been drawn to the possibility of its transmission by the cat,[5] as well as by direct contact with either plague patients having open cutaneous lesions, or with material infected by such patients, or by those with pulmonary or pharyngeal lesions from which the sputum may be infective.

In the primary pneumonic form infection occurs by the inhalation of droplets of infective material produced in the acts of coughing or sneezing by patients with the pneumonic type of the disease. It is known that this method of transmission is common during an epidemic of primary pneumonic plague, and it is a possibility that, under suitable conditions of temperature and humidity, the primary pneumonic type may be similarly contracted from a patient with bubonic plague who has a secondary plague pneumonia. In these cases the infection is said by some to occur in the upper respiratory tract and extend secondarily to the lungs through the blood stream. Others maintain that a primary infection of the lung occurs by direct inhalation of the infective material into the finer bronchioles and air sacs.

[4] Teague and Barber, Ibid. (1912), 7, 172.
[5] Schöbl, Ibid. (1913), 8, 426.

SEPTICÆMIC PLAGUE

While these two types of plague (primary bubonic and primary pneumonic) are universally recognized, other types of the disease have been described by various authors. The Anglo-Indian Plague Commission [6] recognizes four types of the disease: namely, (1) bubonic, (2) septicæmic, (3) pneumonic, and (4) *pestis minor* or *ambulans*. In this classification they refer to a primary plague septicæmia, and present the following description of the type:

Distinguishable clinically though, from the point of view of the pathologist, not sharply marked off from the secondary plague septicæmias just described, are the cases of plague commonly spoken of as septicæmic, in contradistinction to bubonic cases. These are the cases where, owing to the more rapid passage of bacteria through the lymphatic filter, and possibly to a greater production of bacterial poisons, the constitutional symptoms precede and overshadow the local symptoms, the disease being in most cases rapidly fatal.

In another part of the same report we find the following:

Intense or septicæmic type of plague.—In those cases in which the plague virus or toxin is in the patient widespread from the beginning of the illness, so as early to produce a general poisoning, whether septicæmic or toxæmic, the pathological changes, as might be expected, are much the same as in the more severe cases of *Pestis major*. Some observers, however, believe that pathological differences occur to distinguish this form of plague, and to serve, along with the symptoms, as a justification for the establishment of a so-called septicæmic type of the disease. They consist of the absence of buboes having the characters above described, and of a widespread involvement of glands, with distinctive changes in several of them. Although the lymphatic glands are always affected, in place of the affection consisting of one or, more rarely, of several groups of glands being enlarged and surrounded with sero-sanguineous extravasation, while the other glands are either normal or merely enlarged or congested, in this, the so-called septicæmic form, the affection of the glands shows itself as a general involvement of all, or nearly all, of the lymphatic glands of the body, although in many instances the affected glands were chiefly those of the mesentery. In no case, however, did the involvement proceed to the formation of the characteristic plague buboes, but only to a moderate degree of change, practically restricted to the glands themselves, but still displaying in several of them certain distinctive features. These were moderate enlargement and œdema without much congestion, the glands being pink in color, firm and rounded, and with a soft interior, often possessing here and there small areas of softening surrounded by firm substance. Several of the affected glands may be thus modified, while others of them are merely enlarged and engorged with blood, thus resembling the less affected glands of ordinary *Pestis major*. Excepting the lymphatic glands, the parts that were affected showed essentially the same pathological changes as in the

[6] Report of the Indian Plague Commission (1898–99), **5**, 54.

bubonic variety of *Pestis major*, but usually the number of parts affected was smaller and the degree of change in them was less.

From the pathologic standpoint the most detailed description of the so-called septicæmic type of plague is furnished by Childe.[7]

In the bubonic form of plague, one set of glands with extravasated blood around them forms the bubo, and there is practically no alteration in the remaining glands of the body; but in the septicæmic form there is no such bubo, yet there is general involvement of nearly all the lymphatic glands. Yet though so many glands show evidence of disease, one gland or several glands of one set show characteristic changes which are pathognomonic of this type of plague. These appearances are:—The gland is enlarged to the size of an almond or less, is rounded, firm and pink in colour; on section it shows some but not much engorgement and some œdema, its substance is rather soft and can be easily scraped off with a knife, and sometimes small softening areas were present. There was no hæmorrhage in the areolar tissue around this gland and at most only a little œdema and trifling engorgement of the vessels. Commonly there were one or several such glands in one inguinal region, and usually the lowest gland of the chain was most markedly affected; whilst those higher up varied in size from a bean to an almond, and had the same firm pink appearance though there were at times some which looked nearly normal in size and shape. The iliac glands of the same side were similarly affected, as large as almonds and either pink and firm or softer and of a dark red colour. The inguinal glands of the opposite side showed similar changes, but sometimes to a less extent, and the iliac sometimes showed slighter changes or some of them looked normal. The lumbar usually showed slight enlargement and were either pale and soft or somewhat pink and firm. The cervical and axillary varied in size from hazel-nuts to peas and usually showed merely engorgement, being full of dark blood; but sometimes some of them showed the pink firm appearance described above. The mesenteric were enlarged to the size of peas and beans and were either slightly or considerably engorged. The supra-trochlear and popliteal were normal or engorged. There was no hæmorrhage or œdema around any of the above-mentioned glands, and no enlargement of the lymphatic vessels was observed. The condition of the remaining organs was such as has already been described under the bubonic form.

Note.—In several cases of Plague-septicæmia where death had occurred shortly after attack, the glands were found slightly enlarged, of a dark red colour and contained much blood and œdema fluid. This appeared to be an earlier form of the characteristic pink plague glands described above. The difference between the bubonic and septicæmic form of plague appears to be this:—In the bubonic form the plague bacillus after entering the body is arrested at the nearest group of glands, grows here vigorously, and as a result of its growth the bubo is formed. Here the bacillus forms the toxins which are discharged into the system and cause the symptoms of plague, but the glands of the bubo form a barrier which prevents the bacilli from passing on and growing generally throughout the body; and it is, only shortly before death, in fatal cases, that this resistance is overcome and

[7] Report of the Indian Plague Commission (1898–99), 1, 368.

the bacilli are able to pass on into the system generally. But in the septicæmic form the bacillus, after entering the body, meets with feeble resistance at the nearest glands; it speedily overcomes all opposition and passes on to infect other glands and organs where it grows abundantly.

The Austrian Commission also recognizes this type of the disease, which it calls septicopyæmic.

Concerning primary plague septicæmia, the German Commission [8] reports:

Primary plague septicæmia probably does not exist. At least our own Commission as well as the Austrian one, and other investigators, have found on post-mortem examination, in such cases in which the portal of entrance of the virus could not be ascertained, small hæmorrhagic glandular foci, or a focus in the lung. These had in consequence of the indifference of the patients, or in consequence of their occult location, escaped notice during life. Hence plague septicæmia is not a special type of the disease, but the generalization of a primarily local process. That it may then again lead to other secondary internal foci we have demonstrated in a case of plague meningitis.

Strong and Teague,[9] who had the opportunity of studying the epidemic of primary pneumonic plague, reached the following conclusion in regard to primary plague septicæmia:

From our studies made upon human beings, during the Manchurian epidemic, as well as from the animal experiments quoted above, we must conclude that primary plague septicæmia does sometimes take place and that death may occur, though rarely, before visible lesions have taken place either in the lungs or lymphatic glands.

Herzog[10] opposed the classification of plague in man as a general hæmorrhagic septicæmia. This conclusion he bases on "the fact that all observations made on man show that the plague bacillus is not present at all early in the course of the disease in the general blood circulation," and further on the fact that "histologic examinations have further demonstrated that as a rule plague bacilli are either found not at all in the vascular system or are present in such very small numbers that an agonal or post-mortem invasion suggests itself."

Herzog's classification of plague is as follows:

(1) Primary uncomplicated bubonic plague; (2) primary bubonic plague with secondary septico-pyemia; (3) primary bubonic plague with secondary plague pneumonia; (4) primary plague pneumonia; (5) primary plague pneumonia with secondary septico-pyemia; (6) primary plague septicæmia.

In regard to the presence of *B. pestis* in the circulating blood

[8] *Arb. a. d. kais. Gesundheitsamte* (1899), **16**, 75.
[9] *This Journal, Sec. B* (1912), **7**, 180.
[10] *Pub. Bur. Govt. Labs.*, Manila (1904), **No. 23**, 20.

Schöbl [11] made blood cultures from patients with bubonic plague at periods of from three and one-half to seventy-five and one-half hours before death.   He concludes that:

(1) A severe septicæmia may be present at a comparatively early stage of the disease and for a considerable number of hours before death, and (2) the septicæmia may be of an irregular and fluctuating type.

Further he states:

(1) * * * that positive blood culture was obtained in practically every case that was examined in the febrile stage of the disease, even when buboes or signs of pulmonary involvement had not been detected clinically.   (2) It is evident that *Bacillus pestis* may be found in the circulating blood of the patients even in cases which subsequently recover.

In the evidence concerning the occurrence of septicæmic plague above quoted, there appears much that is indefinite and some that is conflicting.   Hence it is not surprising that there is some confusion as to exactly what constitutes a case of "septicæmic plague," if, indeed, such a category is necessary in the nomenclature of plague.   Strictly speaking, any case of plague in which the organisms multiply in the circulating blood is a case of septicæmic plague, but the adoption of this standard would place all fatal cases of both primary bubonic and primary pneumonic plague in this category.   Therefore it would seem more rational to include in this class only those cases (1) in which septicæmia is evidently an early event (2), those in which gross focal visceral plague lesions occur, and (3) those in which the primary buboes are not prominent.   These cases are the ones which give rise to the greatest difficulty from the clinical standpoint, which fall naturally into a class by themselves in the mind of the clinician, and which present both clinically and anatomically the most unmistakable evidences of septicæmia or septicopyæmia.

All three of these features may not be present in the same case, and therefore all cases in this class may not be of exactly the same type anatomically.

In this class should also be placed those cases in which there is mixed infection; that is to say, those in which more than one variety of organism can be isolated from the spleen after death.

INTESTINAL PLAGUE

Some writers have considered that the gastrointestinal tract may be the portal of entry of the plague bacillus and have dis-

[11] *This Journal, Sec. B* (1913), **8**, 413, 415.

tinguished another type of plague of this class. Wilm,[12] Hossack,[13] and Zuppita [14] have reported such cases, but no case of primary intestinal plague has been unequivocally proved.

Childe [15] says:

* * * That no bubo of the mesenteric glands was ever found; these glands were always examined, and though changes might be found in them, they were always less marked and less distinct than plague glands found in other parts of the body. In short, there was no autopsy which went to show that the plague bacillus had reached the stomach or intestine, e. g., in food, and then infected the mesenteric glands.

However, this does not exclude the occurrence of secondary intestinal lesions in plague, which will be described in my cases.

CUTANEOUS PLAGUE

Cutaneous plague does not present any characteristics entitling it to recognition as a separate entity, and the lesions encountered on the skin will be later described.

PESTIS MINOR

The Anglo-Indian Commission [16] reports on *pestis minor* or *ambulans* as follows:

In addition to the three main types of plague which have been described above (bubonic, septicæmic, and pneumonic), an abortive form of bubonic plague comes under observation. This is technically known as *pestis minor* or *pestis ambulans*. It cannot be doubted that in these abortive bubonic cases the bacteria are, as in the case of ordinary bubonic plague, carried to the lymphatic glands, but they are held back there, the disease stopping short of the septicæmic stage. In correspondence with this the constitutional symptoms are very light. Indeed in certain cases not only the constitutional, but also the local symptoms may be so slight as to be, except for their pathological interest, almost undeserving of attention. Such cases appear to be extremely common among persons who have been much exposed to the infection of plague and are characterized by sensations of numbness and tingling, or by neuralgic pains, which in many cases are associated with the development of shotty glands in the armpit and the groins. We may, however, remark here that the whole question of *pestis minor* urgently requires to be more fully elucidated.

Since my experience has been gained in the morgue and laboratory, and cases of *pestis minor* are not fatal, I am not in a position further to refer to these cases.

[12] *Hyg. Rundschau* (1897), Nos. 5 and 6 (quoted by Herzog).
[13] *Brit. Med. Journ.* (1900), 2, 1486.
[14] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1899), 32, 268.
[15] Report of the Indian Plague Commission (1898–99), 1, 368.
[16] Ibid. (1898–99), 5, 54.

AUTHOR'S CLASSIFICATION

From a study of my cases and those in the literature it seems sufficient from the pathologic standpoint to recognize only the two main types of plague: namely, the primary bubonic and the primary pneumonic types. These two types appear to me to include all cases of the disease. If it be desirable to subdivide the types in order to emphasize the fact that the alterations and symptoms do not always follow the same course, they may be so subdivided as to give prominence to the features most commonly encountered. This subdivision will be more of value to enable one unfamiliar with the disease to recognize an atypical first case in a community, rather than as serving to indicate any essential difference in the pathologic processes occurring. Such a classification is here suggested.

I. Primary bubonic plague.
    1. Uncomplicated bubonic plague.
    2. Bubonic plague (with early septicæmia or without superficial buboes).
    3. Bubonic plague (secondary pneumonic type).
    4. Bubonic plague (secondary meningeal type).
    5. Bubonic plague (secondary cutaneous type).
II. Primary pneumonic plague.

To follow this classification to its logical conclusion, those types in which focal hepatic or renal lesions are present should also be separated, but this would serve only unduly to complicate the classification, especially as these types do not present prominent clinical differentiating characteristics.

GENERAL DESCRIPTION OF THE PATHOLOGY OF BUBONIC PLAGUE

The lesions of bubonic plague are due to *Bacillus pestis* and its endotoxins. The bacilli are introduced by the bite of an infected rat flea. A small papule may appear at the point of inoculation. The bacilli multiply and pass along the lymphatic vessels to the lymphatic glands into which they drain. The glands act more or less perfectly as bacterial filters and are at the same time usually profoundly altered by the action of the bacilli. These primarily affected glands are spoken of as the primary bubo of the first order, and from these the bacilli pass along the lymphatics to the next proximal glands, producing alterations in these glands which are, as a rule, of a lower grade of severity than in the primarily affected glands. The glands which are infected from the primary bubo of the first order by direct lymphatic continuity are spoken of as primary buboes of the second order. The bacilli, at some time in the disease,

usually, if not always, enter the circulating blood, and other lymphatic glands throughout the body become infected. These glands, infected through the circulating blood, are known as secondary buboes.

The other parts of the body suffer degenerative changes as the result of the action of the bacteria, of their endotoxins, and of the resultant fever.

The action of the bacilli is particularly severe on the walls of the blood vessels, which accounts for the widespread hæmorrhages which take place.

Focal lesions in different portions of the body occur as the result of bacillary emboli; in this way are produced areas of necrosis, focal or larger, in the spleen, liver, and kidneys, and pneumonic foci in the lungs. Extensive cutaneous lesions may also be thus produced. Meningitis occurs in a small percentage of the cases.

A septicæmia probably occurs at some stage of the disease in the majority of the cases and certainly before death in all fatal cases.

In bubonic plague, as well as in other acute bacterial infections, the infective agent may produce its most injurious effects sometimes in one part of the body and sometimes in another; and according to the parts most seriously affected, there may be distinguished several subtypes of the disease. These have already been referred to under the head of Classification.

PORTAL OF ENTRANCE OF THE INFECTIVE AGENT

Entrance occurs most frequently on the skin and less frequently on the mucous membranes.

Dieudonné and Otto [17] say:

> In bubonic plague the portal of entry for the plague bacillus is chiefly the skin. In practice it is usually very difficult to find this portal of entry. In the majority of cases small abrasions of the skin, flea-bites, and insignificant scratch wounds evidently suffice to furnish the bacteria an entrance; indeed even intensive rubbing the skin with fingers or clothes to which pest bacilli are adherent is sufficient to produce an infection. Since the flea while sucking regularly deposits fæces, it is very possible that by scratching the place the pest bacilli present in the fæces of the flea may be rubbed into the small wounds in the skin.

Dieudonné and Otto's statement that in practice it is usually very difficult to find the portal of entry has been corroborated by my experience. In the majority of the cases at autopsy no

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[17] Kolle und Wassermann, Handbuch der pathogenen Mikroorganismen. Gustav Fischer, Jena (1912), 4, 207.

lesion was visible in the area drained by the glands forming the primary bubo which could be designated as the portal of entry. In a few cases small papules looking like insect bites were found, which in sections showed some necrosis of the skin and masses of bacilli, but it cannot be denied that these might have been noninfective bites which formed a *locus minor resistentiæ*, which favored infection through the blood stream.

Castellani and Chalmers state:[18]

The site of inoculation is sometimes marked by a vesicle, the contents of which contain the *Bacillus pestis* in considerable numbers.

Albrecht and Ghon state:[19]

In no single case could we demonstrate with absolute certainty and incontestably the immediate portal of entry of the pest virus.

The skin is a frequent seat of secondary plague lesions, and plague bacilli may also contaminate wounds and abrasions of the skin. Therefore the presence of pest bacilli in a cutaneous lesion, even though the infection occur in the area of skin drained by the glands constituting the primary bubo, is not sufficient evidence to class that lesion as indubitably the primary portal of entry.

The mucous membranes that form portals of entry are those lining the nose, mouth, pharynx, conjunctiva, and the genitalia. The tonsils are a frequent portal of entry in cases of cervical buboes. (See discussion under cervical buboes.)

In 2 of our cases (1969, 2084) there were found lesions on the foot which, judging especially from the histologic appearance, were in all probability primary lesions. The lesion appears to extend, in these cases, from the skin into the subcutaneous tissues, and is characterized by congestion, œdema, necrosis, hæmorrhage, masses of bacteria, leucocytes, and swelling of the endothelial cells. One of these cases will be described in greater detail.

Case 2084.—Over the middle of the fifth left metatarsal bone is a small, pale papule, or blister, of the skin. Section of this shows it to contain a small amount of turbid fluid. This case has a left femoral bubo and cutaneous petechiæ. Microscopic section of the papule shows some œdema of the epithelium and corium. In the corium and subjacent tissue the fibers of connective tissue are separated (œdema). The vessels are distended with blood. There are large zoögleal masses of bacteria between the tissue fibers, about the sweat glands, and in the lymphatic vessels.

[18] Manual of Tropical Medicine. Wm. Wood & Co., New York (1910), 783.

[19] Über die Beulenpest in Bombay im Jahre 1897. Wien (1898), II B, 484.

A few polymorphonuclear leucocytes are present. There is a small hæmorrhagic extravasation in the outer portion of the corium and some necrosis of tissue about this. Nuclear fragments are seen scattered throughout the area. The area of involvement is greater in the subcutaneous tissue than in the epidermis; the bacteria are very numerous and are present throughout the entire lesion. The endothelial cells lining the lymphatics are large and prominent. While this picture does not furnish absolute evidence of primary cutaneous infection, neither does it exclude it, and taken with the other findings in the case, it is considered that this is in all probability the portal of entry.

SKIN

The Austrian Commission [20] states that the most frequent alteration in the skin is the occurrence of multiple hæmorrhages, varying in size from 2 millimeters to several centimeters in diameter. These are embolic in nature, the vessel lumen being occluded by a bacterial embolus, and they are most frequent in cases with abundant bacteria in the blood and spleen. That they are not purely toxic in origin is shown by the fact that when only a few or no bacilli are in the circulation these hæmorrhages are scant or absent.

In my cases hæmorrhages in the skin were not noted as of unusually frequent occurrence, and they were often so small as to escape notice after death, unless attention was drawn to them by the physician who had seen the case during life. Small vesiculopapular lesions were present in at least 8 of my cases, in some being numerous and widespread over the body and in others being localized. The most frequent type is one in which there are small pinhead-sized, conical, pearly nodules surrounded by a hyperæmic zone. Incision into these discloses a turbid fluid which contains plague bacilli in greater or lesser numbers.

Plague carbuncles occasionally occur. These are described by the Austrian Commission as circular, prominent areas, up to 8 centimeters in diameter, with elevated, firmly infiltrated margins. In the center the epithelium is raised as in a blister, which is filled with thick, reddish exudate. If the blister bursts, the contents, with many bacilli, flow out and the dried-up epithelium collapses and lays bare a moist, damp, bright red and yellow spotted and speckled corium forming the base of the ulcer. These may develop from direct extension over a bubo, by infection through the lymph stream, or through the blood stream.

In 5 cases there were lesions of the skin which corresponded more or less closely to the description of the plague carbuncle furnished by the Austrian Commission. In 3 cases these were

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[20] Über die Beulenpest in Bombay (1898), II B, 481.

single, and in 2 cases, double. In 1 case the lesion was situated in immediate relation to a primary bubo, while in the other cases the infection was presumably through the blood.

Case 2623.—The upper arms and back present numerous small, circular, red, slightly elevated foci without suppuration, which measure up to 0.5 centimeter in diameter. In the left axilla is a large dark area measuring 1.5 centimeter in diameter, on section into which there is necrosis of the skin and immediately underlying tissues, with very dark discoloration as though due to hæmorrhage. A similar, slightly smaller area is present over the manubrium sterni.

Case 2993.—On the left shoulder over the supraspinous fossa is a ruptured pustule, 0.5 centimeter in diameter. Surrounding this is a circle of elevated vesicles, one or two of which have been ruptured. These contain reddish serous fluid. Surrounding this is a zone of deep red skin, the entire area measuring 4 centimeters in diameter. The underlying tissue is firm. Just behind the clavicle to the inner side of the skin lesion are two adjacent, softened, hæmorrhagic lymphatic glands about 1 centimeter in diameter. The tissue surrounding these is œdematous and red with numerous hæmorrhages. This forms the primary bubo.

Case 2383.—Over the left buttock just to the left of the lumbosacral articulation there is a slight excoriation of the skin with a broad zone of reddish discoloration about it. On section into this, reddish creamy fluid exudes, and there is found extensive softening and reddish discoloration of the subcutaneous tissue, extending for a depth of 0.5 centimeter. Over the right buttock near the gluteal fold is a smaller and more superficial reddish area, section into which shows slight reddish discoloration of the tissue only; there is no pus at this point. At other portions of the body are seen a few minute, red punctæ, these being most numerous over the arms.

HISTOPATHOLOGY OF THE SKIN

The lesions occurring at the points which were supposed to be the portals of entry have been already described. There remain the hæmorrhages, papules, and carbuncles. The hæmorrhagic lesions consist of simple hæmorrhages in the corium and subcutaneous tissue. This hæmorrhage is small and does not often extend into the epithelial layer. In my cases there were no large diffuse cutaneous hæmorrhages such as have been described in some epidemics. It was these large cutaneous hæmorrhages that caused the disease to be described as the "black death." The papules present a very characteristic picture of necrosis of the skin with leucocytes and bacterial invasion. A detailed description of one of the papules from case 2335 is here given.

Case 2335.—At the point of the lesion the skin is slightly elevated on account of the infiltration to be described. The epidermis at the apex is reduced to about one quarter of its normal thickness, and all traces of epidermal layers have been destroyed. Here the papillæ are lacking, and the epidermis is represented by an almost homogeneous eosinophilic mass with very few nuclei visible. Clefts in this epidermis show masses of

bacilli. The corium and a superficial part of the subjacent tissue have undergone necrosis and are replaced by a mixture of polymorphonuclear leucocytes, bacilli, and nuclear fragments. The bacilli form dark blue masses in strands and globules. It cannot be recognized whether these strands are in lymphatics or blood capillaries. This infiltration extends slightly laterally in the corium, but not deeply. The vessels in the corium just surrounding this zone of infiltration are much engorged.

The histological changes in the carbuncles differ only in degree from those in the papules. The necrosis and loss of tissue is much greater, and the zone of infiltration with leucocytes and bacilli extends more deeply, forming a virtual phlegmon.

LYMPHATIC GLANDS

Lymphangitis between the point of entry of the infective agent and the primary bubo does not occur. The primary bubo occurs in the lymphatic glands draining the area of the skin which forms the portal of entry of the plague bacillus.

The Anglo-Indian Commission [21] points out that the skin surfaces which drain respectively into the glands of the neck, the axilla, and the groin stand to each other approximately as the figures 1:1.8:5 and that there is a striking coincidence between these figures and those which express the relative frequency of the buboes in these situations, which they found to be 1:1.3:5.8. When the portal of entry of the bacillus is situated in the distal parts of the extremities, the popliteal and cubital glands are seldom the seat of the primary bubo. In this disease, as in other similar infections, the infection passes to the groin and axillary glands, although no satisfactory anatomical explanation of this phenomenon has been offered.

The changes occurring in the primary bubo may be very striking. In a well-marked case there is a visible rounded prominence over the site of the glands, which to the palpating hand feels boggy, elastic, and firmer than normal. Individual glands cannot be palpated, and it may be impossible to move the skin over the subjacent structures. The whole mass is indefinitely outlined, merging gradually into the surrounding tissue. Punctate hæmorrhages may be present in the skin overlying the bubo, and in a very small number of cases definite pustules may have formed. The entire extremity, in the case of femoral or axillary buboes, may be œdematous as the result of pressure of the enlarged glands upon the vessels and as the result of lymph obstruction.

[21] Report of the Indian Plague Commission (1898–99), 5, 70.

On section into a primary bubo there will be found a dense subcutaneous tissue which typically exudes a large amount of clear yellowish fluid. A mass of glands will be found which are enlarged sometimes to as much as 5 centimeters in diameter. These glands are conglomerate and hæmorrhagic, as is also the periglandular areolar tissue, thus making the outlines of the glands indistinct. In the early stages the glands are firm and red, while in the later stages they become softened and show yellowish areas. One or several glands may be involved in this mass, and the amount and extent of the œdema and hæmorrhage varies in different cases. Most frequently there are several glands involved, and the tissues surrounding the glands are also very hæmorrhagic and œdematous, this condition involving muscles, vessel walls, and fasciæ (Plate V). Extension along fascial planes may also be a marked feature, as in groin cases, down Hunter's canal, and, in axillary cases, up into the neck. The infiltration of the subcutaneous areolar tissue between the bubo and the skin may be so dense as to make this tissue almost of cartilaginous consistence.

While this description applies to a well-marked case, the changes may be of a much slighter grade, the primarily affected glands being small and showing very little intra- or extra-glandular hæmorrhage, œdema, or necrosis. In some cases the primary bubo consists only of a single gland with relatively slight changes. It is maintained by the Anglo-Indian Commission that this is the type of disease in which an early septicæmia occurs, the bacteria not being held back by the lymphatic glands. The Austrian Commission demonstrated that true suppuration may occur in the primary bubo from the action of the plague bacillus. In my series are instances of suppuration in the primary bubo and in the meninges, in which the plague bacillus was the only infective agent demonstrable.

Schöbl [22] says:

It can be seen from the table that the plague bacilli may not be detected in the enlarged gland at first and that their presence may be revealed only after repeated examination of the bubo. It is also evident from the results of repeated examinations that the plague bacilli disappear from the infected gland in a comparatively short time, as a rule at the time when pus starts to form. Contrary to the findings in patients who died, distinct phagocytosis was noticed in the smears made from the aspirated liquid in those patients who recovered and who had been treated with serum soon after the onset of the disease. It is undoubtedly this process that clears the gland of the infectious agents.

[22] Loc. cit., p. 412.

These primarily affected glands just described constitute the primary bubo of the first order.

The infection passes from these glands along the lymphatic channels to the next proximal glands, which in turn form the primary buboes of the second order. Retrograde metastases may occur along the lymph channels to the glands of the opposite side, and these glands show changes similar to the primary bubo of the second order. The possibility of a double primary bubo must be borne in mind. That this may occur cannot be doubted, and such cases have been recorded. In only one case (2131) of my series did this seem probable among the groin cases, but seven of the cervical buboes were bilateral, and the lesions on the two sides were so similar that it could not be denied that both might have been primary, although it seems improbable that this was so in all of these cases. The glands of the opposite side of the body may also become infected through the blood, in the same manner as the glands in other parts of the body, thus constituting secondary buboes. The changes in the primary buboes of the second order are similar in character to those in the primary buboes of the first order, but less in degree. The involvement of the glands and periglandular tissue in œdema, hæmorrhage, and necrosis is less, and the glands are not as a rule matted together nor so much enlarged. The œdema, especially, is usually much less marked than about the primary bubo of the first order.

The secondary buboes show lesions similar in character to those usually exhibited in an acute infection by the blood stream. They are slightly enlarged and much congested, but seldom show hæmorrhage, surrounding œdema, or much necrosis. We have, however, encountered one case in which suppuration occurred in a secondary bubo.

Table III shows the location of the primary buboes in our series.

TABLE III.—*Location of buboes in 75 fatal cases.*

Gland.	Right.	Left.	Double.	Total.
Femoral	31	24	1(?)	55
Cervical	2	1	7(?)	10
Axillary	2	4		6
Iliac	2		1	3
Popliteal	1			1

From this table it will be seen that cervical, axillary, and femoral buboes in our series occurred in the proportion of 1:0.6:5.5, as compared with the Anglo-Indian Commission's proportion of 1:1.3:5.8.

HISTOPATHOLOGY OF THE LYMPHATIC GLANDS

To trace the morphological changes in the glands from the beginning of the disease to its advanced stage requires a study of many glands in different stages of the disease. As the primary buboes of the second order are infected through the lymphatics and show lesions slighter in degree than those in the primary buboes of the first order, but similar in character, they form the best material for the study of the earlier changes.

The lesion in the glands is essentially a hæmorrhagic inflammation with coagulation necrosis. In the early stages the plague bacilli are present in large numbers and are situated in masses, chiefly in the peripheral perifollicular lymph sinuses. Early there occurs what is practically a "catarrh" of the lymph sinuses. The endothelial cells lining them enlarge and multiply, and many are found lying free in the sinuses intermingled with red blood cells and a granular material. The sinuses are dilated, apparently from œdema. At the same time œdema of the gland causes it to assume a much looser and more open arrangement of its constituent parts, losing its follicular arrangement.

Extreme congestion of the blood vessels is an early event, and changes in the vessel walls appear. They become swollen and lose their normal appearance, becoming more homogeneous and fibrillar in appearance, Small clefts appear in the vessel walls, and the nuclei fail to stain with hæmotoxylin. This appearance is very similar to an œdematous hyaline change. The lining endothelial cells of the vessels become swollen and are often separated from the basement membrane by small spaces. Fibrin appears at the periphery of the lumen and may be seen in some of the spaces in the vessel wall and, later, in the tissues immediately about the vessels. In some cases this fibrin network completely occludes the lumen of the vessel, but its peripheral arrangement is much more frequently seen. In the later stages the vessels may be very much dilated; their walls are thin, and the lumen may be filled up with polymorphonuclear and mononuclear leucocytes, erythrocytes, and fibrin. Bacilli are also frequently found in these thrombi in the late stages.

The blood passes from the vessels into the surrounding tissue, and the gland is so much enlarged and so hæmorrhagic that the scattered remains of the original adenoid structure are difficult of recognition. They appear as small masses of lymphocytes amid the masses of blood cells and bacteria. Necrosis of the adenoid elements occurs and is represented by masses of granular material containing chromatin fragments. The trabeculæ of the gland undergo a change similar to that of the vessel walls; they

become hyaline and loose in structure and finally become unrecognizable. Polymorphonuclear leucocytes may appear in relatively large numbers, and these may contain bacteria. This seems to be particularly true in cases that have lived for several days. In those cases that have shown macroscopic evidence of suppuration the polymorphonuclear leucocytes are especially abundant. The capsule of the gland becomes infiltrated with red blood cells, leucocytes, and bacteria and undergoes a hyaline degeneration with eventual necrosis. The entire process thus extends to the periglandular tissue, where the adipose tissue may be seen to be infiltrated, hæmorrhagic, and necrotic, in the same manner as is the gland itself. Traces of the capsule of the gland can usually be found microscopically. The vessels in the periglandular tissue may show the same changes as those within the gland, and it is not infrequent to find these occluded by leucocytic and bacillary thrombi. In the gland and periglandular tissue large mononuclear cells, probably derived from the endothelial cells, may be numerous. These engulf the bacteria and fragments of other cells. Groups of plasma cells are not infrequent. The nerves in the neighborhood of the bubo, and the walls of the large vessels, may show morphologic changes similar to those described in the gland.

The blood cells which compose the hæmorrhagic mass in the late stages lose their contour and become a more or less solid mass of eosinophilic structureless material. There may be visible shadows of individual cells. This process is one of hæmolysis.

The number of bacilli appearing in large masses in the glands and periglandular structures is frequently enormous. In the earlier stages these appear to be largely confined to the lymph sinuses; but with the breaking up of the structure of the glands, the bacterial masses are scattered throughout—within the gland, in the surrounding tissue, and in the vessels. In autopsy material, post-mortem proliferation of the bacilli has probably occurred.

In the examination of a bubo in the late stages it may be impossible to recognize it as of glandular structure. One may see only hæmorrhage, œdema, bacterial masses, and necrotic material. From such an examination one could not determine whether the necrosis or hæmorrhage is primary, but it would seem in many instances, at least, that the necrosis of the vessel walls appears before the massive hæmorrhages take place.

It is not possible to state accurately the chronological order in which the various changes in the glands occur, but the changes

may be summarized somewhat as follows: First, there is multiplication of bacilli; then follow congestion, œdema, "catarrh" of the lymph sinuses, hyaline degeneration and necrosis of the walls, migration of erythrocytes and leucocytes, necrosis of glandular tissue, hæmorrhage, formation of fibrin in and about the vessels, vascular thrombosis, and similar involvement in the capsule of the gland and the periglandular structures. These changes are essentially those of hæmorrhagic (occasionally suppurative) inflammation, with coagulation necrosis. The chief peculiarity of the plague bubo is the very abundant œdema of the periglandular structures. The lesions that have been described occur in varying grades of intensity and in varying combinations in all plague-infected glands. In some the hæmorrhage and œdema may be the most-marked feature, while in others suppuration may occur. In slightly affected glands the changes may consist only of bacterial multiplication, œdema, congestion, and catarrh of the sinuses. These form the most prominent features in the average secondary bubo, although in some of these there may be slight hæmorrhage and slight necrosis. The involvement of the capsule and the periglandular tissue is usually minimal or absent in secondary buboes.

BUBONIC PLAGUE WITH EARLY SEPTICÆMIA (SO-CALLED SEPTICÆMIC PLAGUE)

The literature on septicæmic plague has been quoted rather fully in the introduction. The evidence as to what exactly constitutes a case of septicæmic plague is rather confusing. Strictly speaking, any case of plague in which the organisms multiply in the circulating blood is a case of septicæmic plague, but the adoption of this standard would place all fatal cases of both primary bubonic and primary pneumonic plague in this category. It would, therefore, seem more rational to include in this class only those cases in which septicæmia is evidently an early event, those in which gross focal visceral plague lesions occur, and those in which the primary buboes are not prominent. These cases are the ones which give rise to the greatest difficulty from the clinical standpoint, which fall naturally into a class by themselves in the mind of the clinician, and which present both clinically and anatomically the most unmistakable evidences of septicæmia or septicopyæmia. All three of these features may not be present in the same case, and therefore all cases in this class may not be of exactly the same type anatomically.

In this class should also be placed those cases in which there is mixed infection—that is to say, those in which more than one variety of organism can be isolated from the spleen after death.

In three of my cases (2092, 2148, 2150) the pneumococcus and the plague bacillus were isolated from the spleen, in another (2125) a streptococcus and the plague bacillus, and in a third (2267) a streptococcus, *Bacillus mucosus capsulatus,* and the plague bacillus. These cases will be referred to again in the description of the lesions in the spleen.

Excerpts from the autopsy reports of three cases in which the primary bubonic lesion was slight will be presented, and one typical case showing the widespread, gross, focal plague lesions will be reported in full. The following cases illustrate the lesions found in cases of early septicæmia.

Case 2125.—Filipino, male, 20 years old. The duration of illness was four days. The inguinal glands are somewhat enlarged, firm, dark, but no hæmorrhages are present in the surrounding tissues. The glands of the left side are slightly more prominent than those on the right. Slight œdema is present on both sides. On section these glands are dark red and present small hæmorrhages. The axillary glands are smaller, discrete, and red, and show no hæmorrhages in or about them. The popliteal glands are small and red. Internal glands show no change. *Bacillus pestis* was found in the inguinal glands and spleen. *Streptococcus pyogenes* was also isolated in culture from the spleen. No other focal plague lesions were found.

Case 2295.—Filipino, male, 15 years old. Duration of illness was stated as one day. Section over the femoral regions reveals pale, firm, discrete, slightly enlarged lymphatic glands. There is no surrounding œdema nor hæmorrhage. The mesenteric and lumbar glands are small, firm, and pale. Section over the axillary lymphatic glands shows them to be slightly enlarged, discrete, firm, and somewhat reddened. They, however, present no hæmorrhages, nor is there surrounding œdema or hæmorrhage. The faucial tonsils are pale, firm, and not enlarged. The superficial and deep cervical glands are not enlarged, but all are deep red; they show no surrounding hæmorrhage nor œdema. At the bifurcation of the trachea is one large hæmorrhagic and much softened lymphatic gland. This case had numerous cutaneous vesicles and extensive, secondary plague nodules in the lungs.

Case 2378.—Filipino, male, 16 years old. The duration of illness was three days. The superficial lymphatic glands are not palpably enlarged. On section over the right groin the tissues are found very slightly œdematous; the glands are not enlarged, but are somewhat red. The glands in the left groin appear unchanged. The glands in both axillæ are red, but there is no œdema surrounding them and they are not softened. The peribronchial, mesenteric, lumbar, and cervical glands are not enlarged. There were no focal plague lesions. *Bacillus pestis* was isolated from the spleen.

Case 1969.—Chinese, male, 32 years old. The duration of illness exceeded three days. Autopsy was performed one hour after death. The body is that of a well-nourished, male Chinese. On the inner surface of the right foot there is a very small incised wound, which represents the place from which cultures were made of a papule suspected of being the point of inoculation. On the left arm, just below the elbow, is a superficial ulcera-

tion of the skin, which looks like a large ruptured vesicle. This was excised for histological examination. There is marked rigor mortis and no external œdema. Slight post-mortem hypostasis is present.

In both femoral regions are marked rounded prominences. Other superficial nodes are not enlarged. On section into the femoral regions the lymph nodes are found somewhat enlarged but discrete. There is little or no œdema of the tissues surrounding these lymph nodes, and neither the nodes nor surrounding tissue are hæmorrhagic. On the right side one lymph node measures 2 centimeters in diameter and the others are somewhat smaller. On section into these nodes they are somewhat softened, rather pale, and show yellowish white centers which appear softer than the peripheries.

On body section there is a moderate amount of subcutaneous fat. The abdominal cavity is free from adhesions and contains but a small amount of fluid. The liver reaches 4 centimeters below the right costal margin. The diaphragm is at the lower border of the fourth rib on the right and the fifth rib on the left.

The *thorax.* Tissues of the superior and anterior mediastinum are dry and pale. The left lung is adherent at its extreme apex by rather firm, fibrous adhesions. The præcordial area is rather small and is covered with fat. The organs of the neck and thorax were removed en masse. The lingual tonsils are rather prominent. Faucial tonsils are small and pale, but show no lesions. The pharynx and œsophagus are normal. The larynx and trachea are pale. The trachea appears rather broad, and in its upper portion near the bifurcation is a gelatinous strand of mucus, which can be pulled out from the large bronchi, forming practically a cast. There is no congestion of the larynx or trachea. The cervical lymph nodes are not enlarged and are pale. The thyroid is small, rather firm, and of a deep brown color.

The *lungs* are voluminous, and the pleura over them is thin. Both lungs show practically identically the same picture. They are completely filled with nodules, which are firm on palpation through the uncut lung. The nodules average about 1 centimeter in diameter and are uniformly distributed throughout the whole lung, being separated from one another by spaces never more than 1 centimeter in width. Through the pleura, in numerous places, superficially placed nodules appear in the form of discrete and conglomerate yellowish white masses. On section the lung cuts with considerable resistance. There is one old pleural scar at the left apex. The cut surface of the lung is red and moist and presents very numerous nodules varying in size from a few millimeters to 1.5 centimeters. These are rather firm on palpation, grayish white, not distinctly circumscribed, and their centers are somewhat softened, so that purulent or necrotic material can be scraped from their centers by the knife. The intervening lung tissue is deep red and shows some of the smaller nodules. Some of the larger nodules have immediately adjacent to them smaller nodules, giving them an irregular outline. There is no definite cavity formation anywhere, and no fibrosis of the lung and no calcification. One large lymph node at the bifurcation of the trachea is anthracotic, somewhat soft, and rather hæmorrhagic. The other peribronchial lymph nodes appear normal.

The *heart.* The pericardium is free and contains a normal amount of clear fluid. There are no ecchymoses. The heart is rather large, the right side being dilated but flabby. The blood is dark and but slightly coagulated.

Aside from the dilatation of the right heart there is no change except in the musculature, which is pale, rather dry, fairly firm, and somewhat glistening on a smooth cut surface. The endocardium shows no change. The base of the aorta is free.

The *spleen* is enlarged to about one and a half times its normal size. The capsule is rather loose and is steel-blue. On the superior surface, through the capsule near the left extremity, a pale, yellowish white area about 7 millimeters in diameter is visible. Two or three similar, pinhead-sized areas are also visible through the capsule. On section the organ is rather soft and the cut surface is of a pale brown color, the lymphoid and interstitial elements being obscured. Section through the pale areas mentioned shows definite abscess formation, the contents being soft and grumous.

The *adrenals* are small and rather thin, the adrenal medulla being scarcely visible.

The *kidneys* are of about normal size. The capsule strips with slight difficulty; the exposed surface is slightly roughened and pale red. A few pinhead-sized, white foci are visible on the surface. On section the consistence is somewhat diminished. The cortices are rather broad, and the glomeruli are fairly prominent. The vascular striæ are indistinct. The parenchyma is pale, soft, and bulging. The pyramids are bluish at their peripheries and paler at their apices. The ureters and urinary bladder are intact.

The mesenteric lymph nodes are not enlarged. The mesentery contains a moderate amount of fat.

The *gall bladder* and bile ducts are normal.

The *liver* is considerably enlarged, and the capsule is smooth, thin, and transparent. Through the capsule are visible numerous pinhead-sized, pale, yellowish white areas. On section into the liver the consistence is about normal. The cut surface presents numerous small, circumscribed, rather soft, pinhead-sized areas and a few larger areas about 1 centimeter in diameter, yellowish white, rather definitely circumscribed, with softened centers and pale peripheries, surrounded by a red zone. The remaining liver is of a brownish red appearance, the central parts of the lobules being darker than the peripheries.

The *stomach* and *pancreas* are normal in appearance.

The lumbar, retroperitoneal, cervical, axillary, epitrochlear, and popliteal lymph nodes are not enlarged.

It is impossible to say from the anatomical findings where the primary bubo was situated, but from the clinical history the right inguinal would seem to be the glands first affected. The corresponding lumbar glands, however, showed no macroscopic lesions such as would be expected in buboes of the second order.

Anatomic diagnosis.—Plague septicæmia; acute inguinal lymphadenitis, bilateral; multiple abscesses of lungs, liver, spleen, and kidneys; acute parenchymatous degeneration of heart, liver, and kidneys; dilatation of right heart; chronic adhesive pleurisy, localized; cutaneous vesicles.

Report on bacteriological examination of specimen taken from this case two days before death. Furnished by Doctor Schöbl, of the Bureau of Science.

The right femoral bubo is aspirated.

(1) Smears made from the aspirated liquid showed a number of plague-like bacilli.

(2) Cultures made from the said liquid showed pure culture of *B. pestis bubonicæ.* They were Gram-negative, nonmotile bacilli. Agglutination with antiplague serum was positive.

(3) Two guinea pigs were inoculated with the liquid. They showed considerable swelling at the place of inoculation on the third day; also, the enlarged inguinal glands were palpable. One animal died on the third day; the other, on the sixth day after inoculation.

(4) A maculopapulous efflorescence located on the inner part of the right *planta pedis* was incised, and the edges of the wound were scraped; the material so obtained was used to inoculate agar tubes.

(5) Culture obtained therefrom proved to be *B. pestis,* having all the characteristics of *B. pestis* including agglutination.

(6) One guinea pig was inoculated with the material subcutaneously. In three days considerable swelling was noticeable at the place of inoculation. The inguinal glands were swollen and tender. The animal died of plague on the fifth day after inoculation.

History of case 1969.—Ting Nu, 32 years, male, barber by occupation. This case was admitted to the Philippine General Hospital on August 10, 1912. Had fever, femoral bubo, evidence of congestion or some pneumonia, and bloody sputum. He was transferred to San Lazaro Hospital as highly suspicious of plague. While in San Lazaro Hospital he had distinct buboes in both femoral regions, some cough, no bloody sputum, but evidence of some pulmonary complication, delirium, restlessness, and high fever. The glands aspirated on the right side, and organisms morphologically like the plague bacillus were found; also, pure culture was obtained. Animals were inoculated.

This patient had, on the left forearm, a vesicle which he claimed came from a burn, and on the right foot on the inner surface a small papule apparently containing a minute quantity of serum or pus, which it was thought might have been a flea bite and possibly the seat of the primary infection. This did not look, however, like a so-called plague pustule. Some material was taken from this by the bacteriologist.

The case died the morning of August 13, having been sick, according to the history obtained, about seven or eight days.

This case received about 30 cubic centimeters of plague serum, and his condition after receiving it seemed to be improved.

This case exemplifies well that class of cases which should be designated "bubonic plague with early septicæmia." The primary bubo was not a prominent feature, whereas the evidences of septicæmia were predominant from both the clinical and anatomic aspects. The secondary focal visceral lesions are well shown in this case (Plates I and II).

FEMORAL BUBOES

Femoral buboes occur more frequently than buboes in any other situation, this being explained by the Anglo-Indian Commission by the fact that a much larger area of skin surface is drained by these glands than by any others.

Fifty-five femoral buboes were encountered in my series, con-

stituting 73.3 per cent of the total. Twenty-four of the buboes were on the left side and 31 on the right. In 1 case (2131) there was apparently a primary bilateral bubo, and in 4 cases (2072, 2080, 2085, 2131) the inguinal rather than the femoral glands were the seat of the greatest changes. However, as a rule, the femoral and inguinal glands were coextensively involved, and they have been classed, in general, as femoral buboes. In a typical case of femoral bubo there is a visible, rounded prominence over the site of the glands, which in this case is most frequently just below the middle of Poupart's ligament. This swelling may, in some cases, be so slight as not to be readily appreciable to the eye, but in these cases palpation of the two groins will usually reveal a difference manifested by a greater firmness, greater fullness, and greater elasticity over the affected glands. It is usually impossible to differentiate the individual glands by inspection or palpation or to move the skin over the subjacent structures. Punctate hæmorrhages may be present in the skin overlying the bubo, and in a very few cases definite pustules may have formed. (The application of vesicants or caustics over the bubo is a frequent practice among the Filipinos and Chinese.) The entire extremity of the affected side may present an œdematous condition. On section over the glands there is encountered the appearance described on page 265 in the general description of the lymphatic glands. A single gland or all of the femoral and inguinal glands may be involved in the change, and the femoral glands are usually more extensively involved than the inguinal. The œdema about the glands frequently extends along the fascial planes well down into Hunter's canal. The amount and extent of the periglandular œdema and hæmorrhage varies from scarcely perceptible amounts about a single gland to a diffuse, widespread involvement of all the neighboring structures. Typically, a mass of enlarged glands will be found, usually lying along the femoral vein. These glands are enlarged sometimes to as much as 5 centimeters in diameter. They are hæmorrhagic, as is also the periglandular areolar tissue, thus making the outlines of the glands indistinct. In the early stages the glands are firm and red; later, they become softened and show yellowish areas.

Posteriorly to the middle portion of Poupart's ligament is usually found a gland, enlarged to 3 or more centimeters in diameter, which is well encapsulated but very hæmorrhagic and sometimes softened. Extending upward from this, the lymphatic glands along the iliac vessels are usually enlarged, hæmorrhagic,

and sometimes softened.   The hæmorrhage and œdema about these glands are generally not so marked as about the glands forming the primary bubo of the first order; but the hæmorrhage and œdema about the ureter and lower pole of the kidney of the affected side may be very extensive.   The involvement of the glands may extend upward along the vertebral column, affecting all the lumbar glands as far up as the cœliac axis, and may extend across the vertebræ, involving the glands of the opposite side. The peritoneum overlying these glands may show punctate, or larger, more diffuse hæmorrhages.   Frequently the serous surface of the sigmoid flexure of the colon lies in apposition with the peritoneum covering the iliac glands and becomes the seat of an extensive hæmorrhagic condition.   Retrograde metastases may occur along the lymph channels to the groin opposite the original bubo, and the glands here may show changes similar to those of primary buboes of the second order.   The glands of the opposite side may also be infected secondarily through the blood stream.

The Anglo-Indian Commission maintains that the type leading to early septicæmia is that in which the glands forming the primary bubo of the first order show very little enlargement and but little intra- or extraglandular hæmorrhage or œdema. In this instance the bacilli are said not to be held back by the lymphatic glands.   Cases have been encountered in my series in which the femoral and inguinal glands showed slight or no changes, while the iliac glands showed the changes usual in primary buboes of the second order.   Those cases in which no changes were recognizable in the femoral or inguinal glands will be referred to under the heading of "iliac buboes."

The Austrian Commission demonstrates that true suppuration may occur in the primary bubo from the action of the plague bacillus.   In my series are instances of suppuration in the primary bubo and in the meninges in which the plague bacillus only was demonstrable.   Abstracts of the records of these cases follow.

CASES OF SUPPURATION OF BUBOES

Case 2086.—Filipino, male, 37 years old. The duration of illness was seven days. This was anatomically a typical uncomplicated case of bubonic plague with a right femoral primary bubo. The description of the bubo is as follows: The right femoral region shows slight bulging. On section over this region a large œdematous mass is disclosed, which includes one large and several small lymphatic glands. The largest is about 3.5 centimeters in diameter, reddish gray, and very soft and necrotic. The smaller glands are red and hæmorrhagic, but not much softened. There is very little

hæmorrhage in the tissue surrounding the glands. Just beneath the middle of Poupart's ligament are two lymphatic glands which are about 2 centimeters in diameter. These are pale and on section are seen to contain a considerable amount of greenish pus. Smears from the primary bubo show many pest bacilli; smears from the pus show very, very few pest bacilli and no other organisms.

Case 2134.—Filipino, male, 5 months old. The duration of illness was fifteen days. This was anatomically a typical uncomplicated case of bubonic plague with primary cervical bubo. The description of the bubo is as follows: The right side of the neck, just back of the sternocleidomastoid muscle, shows a slight enlargement over an area measuring 2.5 by 2 centimeters. The apex of this is soft but not fluctuating. On cutting through the skin, the knife encounters in the subcutaneous tissue a small amount of purulent reddish gray fluid, which seems to be the substance of broken-down lymphatic glands. The glands in this region are all enlarged, extending upward behind the angle of the jaw and downward and outward behind the clavicle. The glands are for the most part discrete, but swollen and congested. Smears from the purulent fluid show no organism other than *B. pestis*.

Case 2431.—Filipino, female, 19 years old. The duration of illness was two weeks. This was a case with suppuration in the left axilla and an intense suppurative ependymitis. In the right axilla are several glands which are slightly enlarged and moderately hyperæmic but not hæmorrhagic. In the anterior part of the axilla two or three small cavities containing thin grayish pus are opened. It is difficult to say that these abscesses have arisen in the lymphatic glands, though one or two have that appearance. There is slight œdema of the fatty tissue in the anterior part of the axilla, but there are no hæmorrhages. No other glands appearing like primary buboes were found. The cerebral leptomeninges contain a slight excess of fluid, which appears slightly turbid. On opening the right lateral ventricle, it is found to contain a considerable amount of yellowish gray pus. The choroid plexus is gray and soft. The left choroid is smaller, but is also surrounded by grayish exudate. The fourth ventricle is apparently free. The brain substance is pale and shows nothing abnormal. In smears from the spleen, glands, and pus from the axilla no bacteria were found. Smears from the pus of the ventricle were loaded with plump bacilli, which varied greatly in size. These were shown by culture and animal inoculation to be plague bacilli.

Case 3129.—Filipino, male, 6 years old. The duration of illness was one week. This case was one with extensive pharyngeal and laryngeal involvement and pulmonary infarcts, with suppuration in the mesenteric and cervical lymphatic glands. There was a mass of suppurative glands at the head of the pancreas, and some of the cervical prevertebral glands were suppurative. The portal of entry of the bacilli in this case was apparently either the tonsils or pharynx. A few plague bacilli were found in smears from the suppurative glands, and a pure culture was obtained from the spleen.

Case 3215.—Chinese, male, 16 years old. The duration of illness was four days. This was a case with primary left femoral bubo, which developed a large secondary cervical bubo and lobular pneumonia while under observation. The extensive primary bubo had undergone marked necrosis and suppuration in its central portion, and in smears from this pus many plague bacilli were found, a number of which were intracellular.

Case of primary bilateral buboes.—The following case (2131) has been interpreted as one of probable bilateral primary inguinal buboes, as glands on both sides showed the lesions of primary buboes of the first order, and the iliac and lumbar glands on each side showed the lesions characteristic of primary buboes of the second order. Filipino, male, 35 years old. The duration of illness was seven days (?). A single hyperæmic but not enlarged lymphatic gland is found in the right popliteal space. In opening up the right inguinal region, the subcutaneous tissues are found to be very moist, while just below Poupart's ligament is the greatest swelling. On dissecting away the œdematous fat, a chain of enlarged lymphatic glands is found parallel to the ligament. These are surrounded by hæmorrhagic fatty tissue. On section about four or five glands are found to be involved, the largest being 2 centimeters in diameter. They are hæmorrhagic and spotted with numerous small, bright red areas and a few yellowish softened areas. The femoral glands are very slightly, if at all, enlarged and are rather pale. In the left inguinal region the fatty tissues over Poupart's ligament are also slightly œdematous, and one enlarged hæmorrhagic inguinal gland surrounded by hæmorrhagic fatty tissue is found; the other inguinal and the femoral glands on the left side are slightly hyperæmic, but not hæmorrhagic or softened. The iliac and lumbar glands on both sides are much enlarged and are hæmorrhagic, while about the glands and iliac vessels there is much diffuse hæmorrhage in the tissues.

PRIMARY (?) ILIAC BUBOES

Extensive hæmorrhagic lymphadenitis was present in the iliac glands in three cases (2024, 2989, 3194) in which no popliteal, femoral, nor inguinal bubo was recognizable at the time of autopsy.

In the first case (2024)[23] there were also extensive changes in the lumbar, mesocolic, and mesenteric glands and extensive gastrointestinal hæmorrhages. Cultures of *B. pestis* were obtained from the iliac glands, spleen, and lungs. The duration of illness in this case was not recorded.

In the second case (2989) there was found a slightly enlarged, right femoral gland which was firm and red, but was without apparent hæmorrhage. The right iliac glands were much enlarged, hæmorrhagic, and friable, with extensive hæmorrhages about them, extending along the lumbar region as far as the cœliac axis. This case had also extensive secondary pulmonary involvement, and the duration of illness was stated to be ten days.

In the third case (3194) there was no visible change in any of the superficial lymphatic glands, but there was found a mass or chain of enlarged, very hæmorrhagic glands, situated along the right iliac crest and in the right lumbar region to about 5 centimeters above the bifurcation of the aorta. There was no

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[23] This case is reported in full on page 296.

œdema nor infiltration of the tissues surrounding these glands, and they were more or less conglomerate and of firm consistence, showing numerous yellow foci on the red background. *Bacillus pestis* was found in the iliac glands and spleen in large numbers. No other primary focus was found. The duration of illness in this case was said to be one day.

According to Piersol[24] the iliac nodes receive afferent vessels from the bladder and prostate gland, from the lower part of the uterus and the upper part of the vagina, and from the glans penis and clitoris. If the portal of entry of the bacillus be in any of these parts, the iliac glands may form the primary bubo, thus accounting for some of the reported cases in which no bubo was recognizable clinically. The possibilities are that the femoral or inguinal glands, from which the infection spreads to the iliac glands, may never have undergone extensive structural changes; or, on the other hand, they may have recovered from their more severe changes before death occurred.

<div align="center">POPLITEAL BUBOES</div>

In the reports of the German, Austrian, and Anglo-Indian Plague Commissions I have been able to find no report of an autopsy of a case with popliteal buboes, although it is recognized that these occur clinically. The popliteal and cubital glands are seldom the seat of the primary bubo, the organisms in this disease, as in other acute infections, originating in the extremities, passing to the glands in the groin or axilla.

One case of this series had a primary popliteal bubo, and an excerpt from the autopsy record is here presented. These glands may be the seat of secondary buboes, which become infected through the blood stream, and it is said that they may constitute primary buboes of the second order, infected by a retrograde passage of the organisms through the lymph stream.

Case 2081.—There is a considerable bulging in the right popliteal and in the right femoral regions, and in the popliteal region there is found considerable œdema of the tissues. About the deep vessels between the two layers of the gastrocnemii are some much enlarged, deep red, softened, hæmorrhagic glands. The tissues about these are slightly hæmorrhagic, and the œdema extends up through Hunter's canal. On section over the right femoral region the œdema is very great, and there is a large mass of œdematous fat inclosing enlarged lymphatic glands, one of which measures 3.5 centimeters in diameter. This is reddish yellow and soft, and there is considerable hæmorrhage in the tissues about this gland. The

[24] Human Anatomy. J. B. Lippincott Company, Philadelphia and London (1907), 984.

other superficial lymphatic glands are somewhat enlarged, but not hæmor-
rhagic nor œdematous.

In this case both popliteal and femoral glands appear in the gross like
primary buboes of the first order.[25]

AXILLARY BUBOES

When the primary bubo affects the axillary glands, the swell-
ing may be high up in the concavity of the axilla or lower on the
thoracic wall at the margin of the pectoral muscles. In the
former condition the axillary space may be completely filled up
by a large, elastic swelling through which individual glands can-
not be palpated. If the swelling be lower, it will be seen along
the anterior border of the axilla as a diffuse swelling, some
œdema extending in all directions about it. On section the glands
and periglandular tissue will be found in the same condition as
has been described in the groin. The œdema not infrequently
extends upward along the muscle fascia beneath the clavicle, as
far as the postpharyngeal wall, and this œdema may be very
evident in the pharynx. The cervical prevertebral glands may
show the lesions of a primary bubo of the second order. These
will be further discussed along with the primary cervical buboes.

The enlargement of the axillary glands may lead to a diffuse
œdema of the upper extremity of the affected side, as the result
of pressure upon the vessels.

Among my cases 6 presented axillary buboes—2 on the right
and 4 on the left. The liability of the deep cervical glands to
involvement as primary buboes of the second order, with infection
spreading from them more readily to the lung, would seem to
render the lungs more liable to specific plague infection in the
case of primary axillary buboes than is the case with primary
buboes in the groin. This a priori hypothesis is shown to be
true in our cases, as 50 per cent of the cases with primary axillary
buboes had specific pulmonary involvement as compared with
about 7 per cent of the cases with primary buboes in the groin.
These figures must not, however, be accepted without due
allowance for the disparity in incidence of buboes in the axilla
and groin.

CERVICAL BUBOES

Flexner[26] says:

The buccal mucous membrane forms one of the portals of entry into
the body of the plague bacilli. It is probable that the cervical buboes arise

[25] This case is almost exactly analogous to case 18/XLVIII of the Austrian
Commission [Über die Beulenpest in Bombay (1898), II B, 315], in which
cubital and axillary glands were similarly involved.

[26] *Am. Journ. Med. Sci.* (1901), n. s. 122, 405.

from that source of infection. Of all the buccal structures the tonsils seem to be most frequently the primary one attacked. In this fact we have only another illustration of the importance of incomplete epithelial investment and perhaps of previous disease in promoting infection. Other parts of the buccal cavity may become secondary points of development of the bacilli.

The Anglo-Indian Commission [27] reports:

The question as to whether there are channels other than the skin through which the plague bacillus effects an entrance into the system may unhesitatingly be answered in the affirmative. In favour of the view that the infective material may in some cases obtain access to the system through the mucous membranes of the nose, mouth, or pharynx, are: first, the fact that the infection in plague can, in animals, be experimentally produced by the inoculation of the plague bacillus on the mucous membrane of the nose; secondly, the fact that in man buboes under the chin and about the angle of the jaw are not uncommon; thirdly, the fact that the plague bacillus has been found in the human patient in association with primary inflammatory lesions of the tonsils and of the mucous membrane of the nose and the pharynx; lastly, certain epidemiological facts appear to speak in favour of the possibility of the bacillus entering the system by means of the mucous membrane of the nose, such as the fact that, in certain places, epidemics which have been diagnosed as epidemics of mumps preceded and may possibly have stood in casual [?] relation to epidemics of plague. Again, in one instance at least, there is, as we shall see hereafter, reason to suspect that an epidemic of severe coryza may have stood in causal association with a subsequent epidemic of plague.

Strong and Teague [28] have shown that in guinea pigs cervical buboes sometimes may result from the inhalation of a suspension of virulent pest bacilli, and that local application of the bacilli to the tongue or pharynx of monkeys may produce cervical buboes.

In human beings primary cervical buboes may also result from infection of the cutaneous areas drained by the cervical glands.

In this series primary cervical buboes occurred 10 times. In 2 of these cases the bubo was on the right and in one case on the left. In the other 7 cases it appeared to be double, or, at any rate, both sides were involved, and it was impossible to tell on which side the bubo was primary. The glands involved were sometimes superficial at the angle of the jaw and sometimes were deep prevertebral or perilaryngeal glands. The involvement of the prevertebral glands as primary buboes of the second order with primary axillary buboes has already been mentioned. There is usually much œdema associated with primary cervical

[27] Report of the Indian Plague Commission (1898–99), 5, 71.
[28] *This Journal, Sec. B* (1912), 7, 173.

buboes, this extending into the loose tissues about the pharynx. As a result 8 of the 10 cases with primary cervical buboes showed lesions of the pharynx, varying from a simple œdema up to a very marked pseudomembranous pharyngitis and tonsillitis. There seemed to be no method of determining after death, in an individual case, whether or not the glandular involvement was consequent upon primary pharyngeal infection. Careful investigation of the course of events in clinical and experimental cases alone will determine the sequence, and such experience by Strong and Teague has been referred to above.

Our observation that severe pharyngeal lesions, amounting even to a severe grade of tonsillitis, may be secondary to primary axillary buboes suggests that the pharyngeal lesions may also be secondary to cervical buboes when the portal of entry of the infection is on the cutaneous surface. Similar observations were made by the Austrian Commission.

Table IV shows the relation, in this series, of the cervical buboes, pharyngeal lesions, and focal pulmonary plague lesions.

TABLE IV.—*Association of cervical glandular, pharyngeal, and pulmonary lesions.*

Cases with—	Number.	Associated lesions.			
		Cervical buboes.	Pharyngeal lesions.	Focal pulmonary lesions.	None of preceding.
Cervical buboes	10	----------	8	6	1
Pharyngeal lesions	11	8	----------	7	1
Focal pulmonary lesions	13	6	6	----------	5

The relation shown in the table between the cervical buboes and the pharyngeal lesions has already been discussed. The table shows that, of our 10 cases with cervical buboes, 6 had focal plague pulmonary lesions, and that 5 of the 13 cases with focal plague pulmonary lesions had neither cervical buboes nor pharyngeal lesions. An explanation of these will be offered in the part of this article dealing with pulmonary lesions.

PHARYNX AND TONSILS

The lesions of the pharynx should receive consideration in close association with the cervical buboes.

Pathologic conditions of the pharynx are of frequent occurrence in plague. There may be a simple œdema of one part of the pharyngeal wall, or the entire wall may be very much swollen and show various grades of necrosis, hæmorrhage, and ulceration.

The entire pharyngeal ring may be thus involved, or the lesions may be largely confined to the faucial tonsils. In the latter case enlargement, with congestion and the formation of a pseudomembrane or deep ulceration, may be present.

These tonsillar and pharyngeal lesions may represent the reaction of the tissues at the portal of entrance of the bacilli to the body, or they may occur as the result of infection of the parts through the blood stream when the primary portal of entrance of the bacilli is in a distant part of the body. Reference has also already been made to the possibility of the exudate about an axillary bubo extending by direct continuity of tissue to the pharynx. It has also been suggested that these pharyngeal lesions may be secondary to primary cervical buboes when the portal of entrance occurs on the skin surface.

In my series necrotic and ulcerative lesions of the tonsils were present in 3 cases (1894, 2171, 2259) with primary femoral buboes and in 2 cases (2148, 3012) with primary axillary buboes. In the cases of primary buboes of the parotid or submaxillary lymphatic glands (2134, 2150, 2267) the tonsils have not been markedly affected. On the contrary, when the deep prevertebral cervical glands are the ones chiefly involved, severe changes in the tonsils (and sometimes other parts of the pharynx) have been found (2074, 2160, 2335, 2389, 2993, 3129).

These facts would seem to indicate that the tonsils in these latter cases have formed the portal of entrance for the bacilli, and that the involvement of the prevertebral cervical glands occurs secondarily, through the lymph stream.

Plague bacilli are known to multiply rapidly in lymphadenoid tissue, and such tissue forms the major portion of the tonsils. Since, when infected by the plague bacilli, the tonsils undergo changes which are exactly analogous to the changes occurring in the ordinary plague bubo in a lymphatic gland, it is a question whether such primarily affected tonsils may not properly be spoken of as "primary tonsillar buboes." There seems no valid reason why such a term should not be applied to them. There occur in the tonsils congestion, œdema, exudation, hæmorrhage, necrosis, enormous bacterial multiplication, and destruction of the capsule, with involvement of the surrounding structures, and these are the changes that occur in an ordinary plague bubo in a lymphatic gland. On such a basis the prevertebral cervical glands would be called primary buboes of the second order.

From the figures quoted above it will be seen that tonsillar lesions are a not infrequent occurrence in bubonic plague (about 15 per cent in this series).

HISTOPATHOLOGY OF THE PHARYNX AND TONSILS

The changes that occur in the pharynx generally are of a character similar to those in the tonsil, and our description will be confined to the lesions of the latter. The conclusions that were arrived at as the result of the macroscopic examinations are completely confirmed by microscopic examination. In the cases in which the parotid or submaxillary lymphatic glands form the primary bubo, the tonsillar changes are much less severe than in those cases of primary femoral or axillary buboes with tonsillar involvement or in the cases of so-called primary tonsillar buboes. In fact, a moderate grade of congestion, causing some swelling and reddening of the tonsils, is the only change that could be detected in the examination of the tonsils from the 3 cases with parotid or submaxillary buboes.

In the 12 other cases with tonsillar involvement, as noted above, irrespectively of whether the primary bubo was femoral, axillary, or tonsillar, the changes in the tonsil are so extensive that it is practically impossible to follow the changes in their order of sequence. The most that can be done is to state the changes that are found, and their order of sequence will suggest itself by analogy. Congestion of the tonsils in these cases is a constant feature. In the cases which do not show severe changes there also appears to be some active proliferation of the cells in the central parts of the tonsillar follicles. In the later stages all of the internal architecture of the tonsils may be destroyed, so that the follicular arrangement, the sinuses, and the crypts can no longer be oriented. Smaller or larger areas of necrosis occur, containing nuclear fragments, a granular cell detritus, often many bacilli, and usually many polymorphonuclear leucocytes. The bacilli may be present in large masses, and just as abundant as in any primary bubo in a lymphatic gland. Large cells with large centrally placed nuclei, containing in their cytoplasm bacilli and nuclear fragments, may be seen, but these are not frequently numerous. Polymorphonuclear leucocytes may be very numerous and scattered widely throughout the tonsils or may be localized to form abscesses. Not infrequently these cells and lymphocytes may be seen passing through the epithelial layer lining the crypts. Small hæmorrhages may also occur throughout the tonsil. In some cases the cellular elements appear to be separated by spaces in which is a granular material, thus giving the appearance of a loose arrangement to the whole tonsil. This is probably a manifestation of œdema.

Some cases have been seen in which the capsule covering the

lateral and posterior portions of the tonsil has been infiltrated in the same way as the tonsil itself, the œdema, hæmorrhage, and cellular exudate passing for a short distance into the surrounding tissue.

In regard to the epithelium covering the tonsil, sections in some cases may show little, if any, change, even when the tonsil beneath is the seat of severe changes. On the other hand, there may be all grades of infiltration of the epithelium leading to complete necrosis and the formation of a pseudomembrane. This pseudomembrane is made up of necrotic epithelial cells, mucus, leucocytes, red blood cells, fibrin, cellular detritus, and bacteria. Similar masses of granular detritus, bacteria, and leucocytes may be found with the crypts. In cases of less severity the epithelium covering the tonsil may show a simple vacuolation with small collections of bacteria and leucocytes contained within small clefts in the epithelial layer. A not unusual finding is the presence of large masses of bacilli filling a zone immediately below the epithelium covering the tonsil and surrounding pharynx.

It will thus be seen that these changes in the tonsil are remarkably similar to those occurring in a primary lymphatic bubo. The tonsil seldom, if ever, reaches the size of the average lymphatic bubo, but this may be accounted for by the fact of its superficial position and the ease with which the exudate may pass on to the surface.

Attention is here again drawn to the fact that such pharyngeal or tonsillar lesions may occur without specific pulmonary involvement. Therefore the sputum may be infective not only in primary bubonic cases with secondary pulmonary involvement, but also in cases with specific pharyngeal lesions.

<div align="center">RESPIRATORY SYSTEM</div>

<div align="center">LUNGS</div>

Secondary plague lesions occur in the lungs in bubonic plague, and such cases should be distinguished from primary pneumonic plague cases. In the latter class of cases the infection is primary in the respiratory tract, and the pulmonary lesions produced differ very essentially from those that may occur in the course of bubonic plague.

According to earlier studies by Strong, Crowell, and Teague [29]:

* * * it would appear that epidemic plague pneumonia results from inhalation, the primary point of infection being the bronchi. * * * [In the lungs] The bacilli rapidly multiply and produce at first pneumonic

[29] *This Journal, Sec. B* (1912), 7, 220.

changes of the lobular type, and shortly afterwards from the fusion of several rapidly spreading areas more general lobar involvement of the lung tissue * * *. The tonsils may become secondarily infected in pneumonic plague, just as other lymphatic glands—for example, the bronchial ones—become so infected. However, in pneumonic plague death occurs before any very marked macroscopic changes occur in the tonsils. There is no doubt also that the tonsils may become primarily infected in epidemics of pneumonic plague, just as has occurred in sporadic cases during epidemics of bubonic plague.

In many epidemics of primary bubonic plague sporadic cases of such primary respiratory infections occur and are probably to be explained by the same method of transmission as occurs in epidemics of primary pneumonic plague, the contagium in this case arising from the sputum of cases of primary bubonic plague with secondary pulmonary or pharyngeal lesions of the type about to be described. Teague and Barber [30] have offered an explanation, on the basis of temperature and humidity, of the failure of pneumonic plague to become epidemic once a case has occurred during the course of an epidemic of bubonic plague. Their explanation is as follows:

We believe we are justified in concluding from these experiments that were the plague organisms sprayed under similar conditions they would persist longer than cholera vibrios, but a shorter time than prodigiosus bacilli. Hence, it seems probable that the plague bacilli contained in fine droplets of pneumonic-plague sputum would suffer death from drying in a few minutes unless they were suspended in an atmosphere with an extremely small water deficit. Infection in pneumonic plague follows the inhalation of droplets of pneumonic sputum and obviously the longer these droplets remain suspended in the air, the greater is the danger of infection. As has just been stated, these fine droplets disappear very quickly except when they are suspended in an atmosphere with a very small water deficit. Such an atmosphere is under ordinary circumstances of common occurrence in very cold climates, whereas it is extremely rare in warm ones. Hence, since the droplets of sputum persist longer, the plague bacilli remain alive longer in the air, and there is a greater tendency for the disease to spread in cold climates than in warm ones.

The pulmonary lesions in bubonic plague are of three types: the ordinary bronchopneumonic form of the aspiration or hypostatic type, the type of true peripheral infarcts, and a type manifested by few or many nodules widespread throughout the lung which are of metastatic origin. Aside from these focal lesions, varying grades of congestion and œdema of the lungs form a constant feature of bubonic plague. In the first or ordinary bronchopneumonic type of lesion the changes are not macroscopically different from a bronchopneumonia due to other

[30] *This Journal, Sec. B* (1912), 7, 172.

organisms. The description of the lungs in the following case exemplifies the type of lesion encountered in this class of cases.

Case 2073.—Filipino, 31 years old. This case had a typical right femoral primary bubo.

The *lungs.* The pleuræ are deep bluish red throught, and the lungs are slightly nodular on palpation. On section, especially in the posterior part of the lower lobes, are diffuse, slightly elevated, airless areas which are deep red and slightly granular but not redder than the surrounding tissue, which is everywhere deep red and somewhat œdematous. The larger bronchi contain some mucopus, and the mucosa is much reddened. The pulmonary arteries are intact. The peribronchial lymph nodes are red and somewhat softened. At the bifurcation of the trachea are one large and two or three small, very deep red and slightly softened lymphatic glands. The trachea itself has a very much reddened mucosa. The other structures of the neck show no change.

In the infarcted type of pulmonary lesion, also, the changes do not essentially differ from the usual pulmonary infarcts. The infarcts may be single or multiple. In one of our cases (2073) the single infarct was in the upper left lobe; in another (2150) there was an infarct in each lower lobe; and in still another (3129) there were multiple infarcts in the lower lobes.

This type of lesion is exemplified in the lungs of the following case.

Case 3129.—Filipino, male, 6 years old. This case had a cervical bubo probably originating through tonsillar infection, with a severe pseudo-membranous pharyngitis, tonsillitis, and laryngitis.

The *lungs.* The pleuræ are smooth. On the surface of the left lung are found a few small slightly reddened areas, and the tissue beneath these is firm. On section these firm areas have a smooth or slightly granular, grayish red surface, darker in color than the surrounding lung tissue. All are found at the surface and for the most part are more or less wedge-shaped with the bases at the surface. They vary from 0.5 to 3 centimeters in diameter, about 6 being found. The remainder of the lung substance is gray, soft, and moist. The bronchi contain a small amount of mucus. The right lung is similar to the left. The largest nodule is found in the lower lateral edge of the lower lobe. The peribronchial lymph nodes are slightly enlarged and are hyperæmic. No hæmorrhages are seen in the pleura.

In the third or metastatic type of pulmonary lesion the characteristics are exemplified in case 1969 (page 270). The lesions vary in size and number from multiple miliary nodules to single or multiple larger nodules up to 2 centimeters in diameter. These may be very widespread throughout the lungs and occur also on the pleural surface where they cause some elevation of the pleura. The pleura over them shows injection of the smaller vessels, and there may be a delicate layer of fibrin on the pleura over the nodule. The color varies from a pale red to gray or

yellowish gray, and the areas are surrounded by a narrow red hyperæmic zone. The consistence of the nodules is firm in the earlier lesions, and softening occurs in the central part of the older nodules. In the same lung there may be nodules of varying size, color, and consistence. In some cases it is possible to recognize a definite arrangement of these about the bronchi, while in other cases no such arrangement is discernible. The nodules are circular in outline and discrete, and no evidence of any attempt at fusion of the nodules to form a more general lobar involvement has been seen. The sharp delimitation of these areas contrasts with the indefinite outline of the early pulmonary lesions in primary pneumonic plague. The lung substance between these nodules is crepitant, but as a rule congested and œdematous.

It will thus be seen that the lesions in this type of plague correspond to those described in other infections as metastatic or septic embolic pneumonia. However, these lesions never progress to the stage of cavity formation, probably because of the short duration of the disease. These nodules can best be explained on the basis of a metastatic or embolic origin. While the type of true peripheral infarct and the metastatic embolic type of pneumonia are similar from the etiologic and microscopic standpoints, it seems desirable to separate them on account of their macroscopic variations. The term "infarct" suggests at once the idea of a peripherally situated, cone-shaped area of necrosis with possible suppuration, such as is encountered in my second class. When, on the other hand, cases occur with globular nodules found widespread throughout the lung, having no predilection for a peripheral situation, the cases may well be segregated in a class by themselves. From their situation in relation to the bronchi, and from the existence of a true bronchitis in these cases, it may well be that some of them are of bronchogenic rather than hæmatogenic origin.

An attempt has been made to correlate these pulmonary lesions with the existence of laryngeal, pharyngeal, or tonsillar lesions, but all three types occur irrespectively of whether such lesions exist and independently of the site of the primary bubo. My series shows that 5 of the 13 cases with pulmonary lesions had neither cervical buboes nor pharyngeal lesions; that of 10 cases of cervical buboes 6 had pulmonary lesions; and that of 11 cases of pharyngeal lesions 7 had pulmonary lesions. These figures do not give proportions sufficient to justify one in drawing any positive conclusions as to the necessity for a causal relation between these lesions. This doubt as to the causal relation is

enhanced when it is learned that the pulmonary changes spoken of are of both origins—as infarcts and as metastases.

HISTOPATHOLOGY OF THE LUNGS

In the first or purely bronchopneumonic type of lesion the microscopic changes do not differ essentially from the same lesions caused by other organisms. Surrounding the bronchi there occur localized areas in which the alveoli contain some serum, many red blood cells, a few leucocytes, and some large pigment cells. The capillaries in the alveolar walls are engorged, and small hæmorrhages may occur. The tissue immediately surrounding the consolidated area is congested and may show some collapsed alveoli. The bronchi show evidence of a simple catarrhal inflammation. Bacilli are, as a rule, not a prominent feature in sections of lungs of this type. Fibrin in the exudate is very scant or completely lacking.

In lesions of the second and third types (peripheral infarcts and metastatic embolic pneumonia) the leucocytes and bacteria are present in greater abundance, and the destruction of tissue is greater than in the first type. Here one finds foci of very dense infiltration with polymorphonuclear leucocytes and a few erythrocytes. These are enmeshed in a fine fibrillar network. Weigert's stain for fibrin shows some fine strands and networks of fibrin, but this is not nearly so abundant as in a frank lobar pneumonia. In the alveoli are also seen many large pigmented cells, obviously desquamated epithelial cells from the alveolar wall. These cells are found very constantly in the pulmonary alveoli in plague, irrespectively of whether or not there is consolidation present. Bacilli may be present in some of the alveoli in rather large numbers, but in this series they are not nearly so abundant nor so conspicuous a feature as they were in a previous series of primary pneumonic-plague cases. Nuclear fragments may also be abundant in the alveoli.

In the central parts of the nodules the interalveolar septa are represented by diffuse eosin-staining masses, which show no organized structure. In the places of some of these are dense masses of bacilli, which masses have such shapes as to suggest that they lie in vessels, although all traces of the vessels have disappeared. The peripheral parts of these foci show better preserved septa, with engorged capillaries; the septa, however, show the same type of changes as described in the smaller splenic vessels. They are thickened and more or less homogeneous, take the acid stains, and show a fibrillation of their structure.

Over the peripherally placed infarcts there is a delicate layer

of fibrin on the pleura, and this contains a few leucocytes and some bacteria. The endothelial cells beneath the fibrin are seldom recognizable. Just beneath the pleura at these points there is usually a very dense mass of bacilli.

Surrounding the nodules just described there is seen a zone of compressed alveoli with engorged vessels.

Sections of the bronchioles and bronchi show regularly some desquamation of the lining epithelial cells and a mass of cell detritus containing leucocytes and bacteria in the lumen. In the walls of the bronchi there is marked engorgement of the vessels.

PLEURA

The most frequent lesion of the pleura is the presence of punctate hæmorrhages, which may be very few in number or may be numerous and scattered over both the parietal and the visceral layers. Such hæmorrhages were noted in 33 of our cases, these standing, in this series, next in frequency only to those on the epicardium. Localized areas of an acute fibrinous pleuritis may also be present over the peripherally placed infarcts and metastatic foci in the lungs.

BRONCHI AND TRACHEA

Congestion and an acute inflammation may be present in the trachea and bronchi, occurring most often in those cases in which there was specific pulmonary involvement. The inflammation may be of the simple catarrhal or of the croupous type. One case occurred in which a large plug of fibrin and mucus was pulled out of the trachea and bronchi, of which it formed an imperfect cast.

LARYNX

The larynx is most liable to involvement in the case of axillary or cervical buboes in which there is a widespread involvement of the pharyngeal region. Hæmorrhages on both sides of the epiglottis, and œdema, which may involve the aryteno-epiglottidean folds, the epiglottis, and the vocal cords, constitute the chief laryngeal changes encountered. A pseudomembranous laryngitis and pharyngitis was found in two cases (2389, 3129), in one of which (3129) the clinical diagnosis of diphtheria had been considered.

SPLEEN

The spleen in bubonic plague presents a very characteristic appearance, the most characteristic features being its color and its consistence. The spleen is usually enlarged, but not in all cases. The size varies from those which are smaller than normal

up to those nearly three times larger than normal, the average being somewhat above normal. The enlargement takes place in all directions. In an average of 14 spleens of my series that were measured the measurements were 13 by 9 by 4.5 centimeters. The spleens that were measured do not include those of the greatest size, so that these measurements are probably minimal rather than maximal. The average measurements of the spleen given by the Austrian Commission are 12 to 22 by 6 to 17 by 3 to 6 centimeters. These sizes are stated without reference to cases in which the enlargement of the spleen was obviously due to chronic preëxisting disease.

The capsule of the spleen is tense, opaque, and varies in color from a reddish brown to a steel gray. Frequently the capsule presents numerous, small, discrete or confluent hæmorrhages scattered over its surface. The consistence of the organ is firm, and this is as marked after as before transverse section. When placed on the table after removal, the spleen retains its shape, and after section the organ does not collapse nor does the pulp flow out. In this respect the spleen differs remarkably from the acutely enlarged spleen seen in typhoid fever and other septicæmic conditions.

The cut surface of the spleen is a dull grayish red color and is not glistening. Both the consistence and color are remarkably like those encountered in cases of diffuse amyloid disease of the spleen. The cut surface, however, lacks the glistening appearance and is not smooth. It is rather rough and has a shagreen appearance, the pulp bulging out in the form of small, rounded, closely packed masses. As a rule, the trabeculæ, smaller vessels, and lymphoid follicles are not recognizable. Small hæmorrhages, which are darker in color than the surrounding pulp, may be recognizable on the cut surface.

Small infarcts and necrotic nodules are visible to the naked eye in a small proportion of cases, and in these cases there are usually similar lesions in other viscera. In other words, these occur in cases of septicopyæmia. Three cases with splenic infarcts occurred in the series, and their description follows.

Small areas of focal necrosis were also recognizable in a few cases. The color and consistence of the spleen are considered among the most characteristic features occurring in plague. After having become familiar with them, we have ventured the diagnosis of plague even before inspection of the buboes, and even in some cases when the bubo was atypical. Furthermore, after having become familiar with the color and consistence of the spleen in plague, on finding a soft, pulpy, diffluent spleen

of dark color in a case of known plague, the diagnosis of mixed or secondary infection was ventured before bacteriological examination and was later confirmed.   That it is possible to do this is shown by the fact that this was an independent observation of mine, and it is only recently that I have found that this observation was also made by the Austrian Commission.

CASES OF SPLENIC INFARCTS

Cases 2079.—Filipino, male, 34 years old.   The duration of illness was five days.   The anatomic diagnosis was as follows: Bubonic plague; acute femoral lymphadenitis, right (primary bubo); acute iliac and lumbar lymphadenitis (primary bubo of the second order); acute lymphadenitis, left femoral and axillary (secondary buboes); double hydrothorax; congestion, œdema, and atelectasis of lungs; epicardial, endocardial, pleural, gastric, renal, peritoneal, and splenic ecchymoses; congestion and degeneration of kidneys and liver; acute splenitis; infarcts of spleen; trichuriasis.   Very numerous plague bacilli were found in the buboes and spleen.   The description of the spleen is as follows: The spleen is considerably enlarged, measuring 15 by 11.5 by 6 centimeters.   The capsule is steel-blue and tense.   On section the organ is firm and deep red, the cut surface being smooth and showing numerous minute hæmorrhages scattered throughout the pulp.   The lymphoid tissue is not visible.   There is a small, wedge-shaped, peripherally placed, yellow, somewhat softened area.

Case 2993.—Chinese, male, 35 years old.   The duration of illness was three days.   The anatomic diagnosis was as follows: Bubonic plague; acute hæmorrhagic lymphadenitis (left lower cervical); acute pustular and vesicular dermatitis (plague carbuncle); acute hæmorrhagic lymphadenitis (right upper cervical); acute pseudomembranous tonsillitis; acute splenitis; infarcts of spleen and liver; petechial hæmorrhages in epicardium; congestion and œdema of lungs.   The description of the spleen is as follows: The spleen is enlarged to about twice or three times its normal size and is very friable.   The cut surface is moist, uneven, and deep red but not dark.   The Malpighian bodies and the trabeculæ are obscured by the soft swollen pulp.   On the surface are seen a few small grayish areas from 2 to 8 millimeters in diameter.   They consist of opaque friable tissue.

See also the report of the spleen in case 1969 (page 270).

HISTOPATHOLOGY OF THE SPLEEN

Extreme engorgement of the spleen with red blood cells is the most striking feature.   On account of the structure of the splenic sinuses it is difficult to recognize small hæmorrhages histologically, but in bubonic plague the presence of large areas in which nothing but erythrocytes are visible, even in very thin sections, leaves no doubt in the mind that actual hæmorrhage has occurred.   Such hæmorrhages, either at the periphery of the spleen or in its central part, are an almost constant feature of this disease.   The erythrocytes are found in all stages of dissolution.   The endothelial cells lining the sinuses become en-

larged and vacuolated and frequently desquamated. Large cells, which are apparently derived from these, are found lying free in the sinuses. Their nuclei are single or they may be double. Cells no larger than these are also seen with as many as 10 or 12 closely packed nuclei and with very indefinite cytoplasmic structure. The origin of these latter cells was not determined; they may be derived from the endothelium lining the sinuses, or they may be marrow cells. It is not unusual to find considerable numbers of polymorphonuclear leucocytes lying among the cells of the splenic pulp.

The condition of the lymphoid follicles varies. In a fair percentage of the cases they are normal in size, and in no case of this series were they noticeably enlarged. Diminution in size and relative scarcity of the follicles is frequently seen. The central vessel in the follicles is usually engorged, and its walls appear thickened and poor in nuclei with a fibrillated structure which is eosinophilic. The adventitia is loose, and there is frequently a clear space immediately about the vessel. The entire structure of the follicles is loose and open, the lymphocytes being separated from one another. Evidence of hyperplasia of the follicles, as manifested by enlargement and mitotic figures of the cells in the germinal centers, is not frequently seen in plague. Bacilli within the follicles are never numerous. Hæmorrhages from the surrounding pulp may extend into and break up the follicles. In some cases the congestion of the spleen is most marked about the follicles, as has been noted previously in pneumonic plague, but this feature is not so noticeable and not nearly so constant in the spleens of this series.

The trabeculæ show the same evidences of hyaline degeneration and frequently necrosis, as has been described in the walls of the blood vessels and the trabeculæ in the buboes. Small localized areas of hæmorrhagic necrosis occur throughout the spleen in a large proportion of the cases. In these one sees large numbers of erythrocytes with very few other formed cellular elements, but large masses of chromatin particles. Larger infarcted areas were visible to the naked eye in three of our cases, as noted previously. These infarcted areas are rich in bacilli. Plague bacilli are almost always present in the spleen in rather large numbers, but they do not occur in the large zoögleal masses such as are seen in the buboes. They are more widely scattered through the splenic pulp, in the sinuses, and in the endothelial cells. They are sometimes seen

in the polymorphonuclear leucocytes. In one of our cases no bacilli were found in smears, cultures, or sections of the spleen. As a rule, at autopsy smear preparations from the spleen show large numbers of plague bacilli, whereas in appropriately stained sections they frequently appear to be relatively few.

Fibrin formation is not prominent in the spleen in bubonic plague. In a few cases only were fine threads of fibrin found in and about the vessels and sinuses.

The essential lesions in the spleen in bubonic plague then are congestion and hæmorrhage, necrosis, endothelial proliferation, and bacillary infiltration, with degenerative changes in the walls of the blood vessels and trabeculæ.

LIVER

The liver regularly presents the condition of acute parenchymatous degeneration, which does not essentially differ from the same condition encountered in other acute infections.

Hæmorrhages in the capsule of the liver and about the gall bladder are a frequent finding. Small hæmorrhages and areas of focal necrosis are recognizable in some of the livers by the naked eye. Extreme congestion may be present in some cases, and some few present a fairly advanced fatty degeneration. In general, the macroscopic appearances of the liver are not especially characteristic of the disease and do not differ from those found in other acute infections.

Infarcts and nodules similar to those found in the spleen were present in the livers of two of my cases (1969, 2993). An acute cholecystitis was also present in one instance (2074).

HISTOPATHOLOGY OF THE LIVER

The histological changes in the liver are chiefly acute parenchymatous degeneration, congestion, and focal and larger areas of necrosis. Acute parenchymatous degeneration of a moderate grade is a practically constant finding in our cases. Congestion is also of frequent occurrence. This is especially marked in the central parts of the lobules, and the columns of liver cells may be widely separated by the engorged vessels, giving the appearance of a chronic passive congestion. Pigment in the cells, such as is seen in such a condition, is, however, not frequently found. The liver cells show not only the evidences of acute parenchymatous degeneration, but are frequently vacuolated, and the nuclei often lie in a clear space. Foci are seen which seem to be especially in the peripheral parts of the lobules, in which the protoplasm has undergone solution, leaving a spongelike

reticular structure. As a rule, only a few cells are involved in such a focus. The nuclei may remain apparently intact, or they may become fragmented and disappear. These areas of focal necrosis are similar to those seen in typhoid and other acute infections. Hæmorrhages into these necrotic foci sometimes take place. The endothelial cells of the vessels appear swollen, and small fibrin threads are sometimes seen. The areas of focal necrosis can be found in almost every case, if several slides are searched for them. Lymphocytes, and occasionally polymorphonuclear cells, are frequently found in the portal spaces surrounding the portal vessels. Desquamation of the epithelium in the bile ducts in the portal spaces is often seen. The vessels seldom show thrombosis. Bacilli are found, but not in as large numbers as in the spleen or buboes. In a few of the cases nodules like infarcts were found. These show large masses of bacilli amid necrotic liver cells with some polymorphonuclear leucocytes. Vascular thrombosis probably accounts for these changes, but we were unable definitely to associate them with the lesions in our cases.

GASTROINTESTINAL TRACT

Here also the chief lesions encountered are obviously due to the hæmorrhages.

In 30 cases hæmorrhages were noted in the mucosa of the stomach, and in 27 cases in the intestine. Of all hæmorrhages on mucous membranes these are the most frequent. In some cases they are small and widely scattered, while in others they are larger and more closely packed. In the stomach they occur mostly on the crests of the rugæ and here frequently give rise to superficial erosions of the mucosa. These erosions or small ulcers are either circular or linear in outline, following the direction of the rugæ on which they are placed. They are frequently multiple and may be very numerous. The circular ones vary in diameter from 1 to 3 or 4 millimeters, while the linear ones are from 0.5 to 1 centimeter in length. The larger ones frequently have a yellowish base and margins and are placed in the central part of the hæmorrhagic area. Twenty of the cases showed these erosions of the mucosa in the stomach (Plate IV).

A general hyperæmia of the gastric mucosa without hæmorrhages was not infrequent, and in many cases there was an excess of mucus on the surface.

In the intestine any part may be affected, and the hæmorrhages occur with about equal frequency in the small and large intestine. In some cases the entire mucosa of the large intestine may be

very thickly beset with punctate hæmorrhages, so that no square centimeter of the entire surface is free from them. A type of plague has been described by some authors in which the intestine was said to form the portal of entrance for the bacilli to the body. In such cases primary buboes were described as occurring in the mesenteric glands.

Attempts by various workers to reproduce this type of plague in animals by feeding cultures of the bacilli have resulted in primary infection through the mouth or pharynx rather than through the intestine. Similarly, in animals naturally infected by ingestion, cervical rather than mesenteric buboes occur. The Anglo-Indian Commission [31] and the Austrian Commission [32] agree in stating that no case was seen in which alimentary infection was considered probable. In my series three cases occurred in which plague lesions other than hæmorrhages were present in the intestine, but in each of these there were portals of entry in other parts, and both the intestinal lesions and the slight changes in the mesenteric glands were interpreted as secondary infections with the plague bacilli through the blood stream.

In the first case (2125) the infection apparently entered through the tonsil, which showed ulceration and the lesions described elsewhere in this paper as those characteristic of a primary tonsillar bubo. All of the superficial glands showed lesions characteristic of secondary buboes.

In the intestine beginning with the descending portion of the colon, extending through the sigmoid, and most prominent in the rectum, were numerous small ulcers, pin-point to pinhead in size. These were surrounded by a narrow hyperæmic zone. In the descending colon there were many grayish white, pinhead-sized, soft nodules. In the rectum the ulcers were so numerous as to give a hyperæmic appearance to the entire mucosa. The mesenteric glands were red and moist, like secondary buboes. No other focal plague lesions were found in this body. *Streptococcus pyogenes* and *Bacillus pestis* were isolated from the spleen.

The second case (2148) was one with left axillary bubo and a pseudomembranous pharyngitis and laryngitis. The mucosa of the intestine showed no lesions except in the rectum, where there were fairly numerous pinhead-sized, slightly raised, pale, soft foci. Some of these were surrounded by a narrow red zone.

[31] Report of the Indian Plague Commission (1898–99), 1, 368.
[32] Über die Beulenpest in Bombay (1898), II B, 543.

The mesenteric and mesocolic nodes were slightly enlarged, firm, and pink. *Pneumococcus* and *B. pestis* were isolated from the spleen.

The third case (2024) will be reported in full.

These cases form no basis for assuming a primary intestinal infection, but on the other hand prove that these intestinal lesions may occur secondarily.

The intestinal lesions contain large numbers of bacilli, which would make the fæces of these cases a source of contamination.

Intestinal parasites (*Ascaris lumbricoides, Trichuris trichiuris,* and hookworms) were found in 46 of the 75 cases. In 8 of the cases all 3 species were found, in 18 cases 2 species were found, and in 20 cases only 1 species was found.

CASE WITH EXTENSIVE GASTROINTESTINAL HÆMORRHAGES

Case 2024.—Filipino, male, 30 years old. Anatomic diagnosis: Bubonic plague; acute hæmorrhagic lymphadenitis, iliac (primary bubo), mesocolic, mesenteric, and lumbar; retroperitoneal ecchymoses; hæmorrhages, epicardial, pleural, peritoneal, intestinal, and gastric; acute gastritis with hæmorrhagic erosions; congestion of lungs, kidneys, and liver; parenchymatous degeneration of kidneys and liver; acute hyperplasia of spleen.

The *body* is that of an adult, male Filipino which is well-nourished and exhibits marked rigor mortis and some post-mortem suggillation. The body is still warm. There are no scars or cutaneous abrasions. The superficial lymphatic glands are not markedly enlarged. The pupils are equal, moderately dilated, circular. The conjunctivæ and corneæ are clear. The nose, ears, and mouth are normal. On section there is a moderate amount of subcutaneous fat. The muscles are well-developed, brown, and moist. The abdominal cavity is free from fluid and adhesions. The initial body section was continued into both inguinal regions, where no œdema was found; the lymphatic glands were not enlarged. The intestines are moderately distended, and the peritoneum along the brim of the pelvis shows diffuse bluish discoloration with minute petechiæ. The liver reaches the right costal margin. The diaphragm is at the fourth interspace on the right and fifth interspace on the left.

The *thorax.* The pleural sacs are free from fluid and adhesions. There is a small amount of thymic tissue which is pink and glandular, the thymic tissue probably not exceeding 5 grams in weight. The tissues of the anterior mediastinum are not œdematous. The lungs are somewhat retracted, exposing a large præcordial area. On opening the pericardium, there is a slight excess of clear fluid. The parietal pericardium is smooth and pale.

The *heart* is large; the apex is formed by the left ventricle. The right ventricle presents anteriorly. There are numerous petechiæ over the epicardium, especially on the right side of the heart. The right heart contains a considerable amount of red fluid and clotted blood. The tricuspid ring admits three fingers. The endocardium is smooth and pale throughout the right heart. The tricuspid and pulmonary leaflets are thin and pliable. The muscle of the left ventricle is considerably thickened, dark, and moist. The

left heart is empty. The mitral ring admits two fingers. The endocardium throughout is smooth. The mitral and aortic leaflets are thin and pliable. The base of the aorta is free. The muscle of the left ventricle is dark, moist, and firm and not hypertrophied. The coronary arteries are intact.

The *lungs* are somewhat diminished in volume, the pleura over them being smooth but showing numerous petechiæ over both lungs. The lungs are crepitant, and their elasticity seems diminished. On section of the lungs the cut surface shows the same appearance anteriorly and posteriorly in both superior and inferior lobes. This consists of a moist, dark red, smooth surface which exudes some bloody, frothy fluid on pressure, but this is not large in amount. The bronchi have a bluish purple mucosa and are empty. The pulmonary vessels are free. The peribronchial lymph nodes are small, deeply pigmented, and firm.

The *neck organs.* The faucial tonsils are very slightly enlarged, but show no gross changes. The lingual follicles are very prominent and pale. The tissues of the pharynx have a normal appearance, being perhaps slightly congested. The larynx and trachea have a normal appearance. The œsophagus is normal. The cervical lymphatic glands are not enlarged. The thyroid is normal in appearance.

The *spleen* is very considerably enlarged (to about twice the normal size). The capsule is rather tense. On section the organ is of good consistence, and the cut surface is of an opaque, grayish red appearance, the pulp being rather prominent. The lymphoid and interstitial elements are obscured. There are no visible hæmorrhages. The cut surface, while firm, is not absolutely smooth.

The *adrenals* are of normal appearance.

The *kidneys* are somewhat enlarged. The capsule strips readily and shows no hæmorrhages. The surfaces of the kidneys are smooth and very deep red. The consistence of the organ is somewhat increased, and it is rather resilient. The cut surface shows deep congestion of both cortex and pyramids with few rather diffuse, paler areas in the cortex. These are rather large and not well circumscribed. The vascular markings are rather prominent, but the glomeruli are not readily seen. The pelves and ureters are intact.

The *urinary bladder* is distended with a clear urine. Its mucosa is pale. The prostate is normal.

The *intestines.* The small intestine, from the duodenum to the ileocæcal ring, contains some dark flecks of coagulated blood and a small amount of dark, semiformed fæcal material. The mucosa throughout shows numerous minute petechiæ, but there is no suggestion of any membrane and no ulceration. The large intestine from the cæcum to the rectum is the seat of still more numerous petechial hæmorrhages, these all being isolated and minute but very closely packed throughout the entire intestine. Here, also, there is no evidence of any inflammatory reaction. The mesenteric and mesocolic lymphatic glands are prominent on account of their color, these being deep red, but they are not enlarged and show no evidence of necrosis nor surrounding œdema.

The *gall bladder* and *bile ducts* are normal.

The *liver* is slightly enlarged; its capsule is thin and smooth and is without hæmorrhages. On section the cut surface is smooth, brownish red, and slightly opaque with fairly distinct lobular markings.

The *pancreas* is normal in appearance.

The *stomach* contains some blackish flecks of coagulated blood over the

mucosa. On removal of this there are found very numerous petechiæ in the mucosa. In addition, there are numerous excavations mostly circular in outline which are not deep and do not extend beneath the submucosa; their bases are flat and very deep red in color, and the mucosa immediately surrounding them is also very deep red in addition to presenting the petechial hæmorrhages. The cardiac end of the œsophagus is absolutely normal in appearance, and the sharp demarcation of the congestion and hæmorrhages which begins at the cardiac orifice is very striking.

The *retroperitoneal tissues*. On dissecting up the peritoneum from the course of the iliac vessels and the aorta, there is found a diffuse hæmorrhagic condition which extends up as high as the cœliac axis and outward over the poles of both kidneys. On dissection into this hæmorrhagic tissue there are found numerous, enlarged, softened, and hæmorrhagic lymphatic glands, some of which show small yellowish areas. The tissues between these lymphatic glands are so hæmorrhagic as to make the differentiation between the lymphatic tissue and intervening tissue rather difficult. This completely surrounds the abdominal aorta, which is of a very small caliber; its endothelium, however, is smooth.

The *head*. The structures of the scalp and calvarium are normal. The dura mater is nonadherent and is rather bluish externally and pale and smooth internally. There is no excess of fluid in the meninges over the hemispheres or at the base. The meningeal vessels are filled with blood. The ventricles are not distended. The ependyma is smooth. Section of the brain substance reveals no gross lesions.

Section into the axillæ shows normal lymphatic structures.

The report on bacteriological examination of material obtained by autopsy was made by Doctor Schöbl, of the Bureau of Science. Specimens subjected to examination consisted of:

(1) Smears from the retroperitoneal hæmorrhagic glands, from the spleen, and from the lungs.

(2) Cultures on agar slants made at the autopsy.

All smears revealed numerous typical plague bacilli (Gram-negative).

Cultures from the spleen: Scanty growth was noticed on the surface of the culture medium at the end of twenty-four hours' incubation. Smears were made from the water of condensation. They showed typical bipolar-staining bacilli arranged in chains. In forty-eight hours small colonies developed on the surface, showing typical shape and elevated, granular center, with thin wavy margin. The culture was agglutinated by antiplague serum.

Cultures from the glands: Cultures were slightly contaminated. Subcultures were pure and typical for plague.

Animal experiment: Owing to the purity of the spleen cultures one guinea pig was infected by subcutaneous, another one by intraperitoneal infection. They both died on the fourth day after inoculation and exhibited typical lesions in the inguinal glands at the place of infection and in the spleen. Upon microscopical examination of the smears made from the bubo, spleen, and blood of the diseased animals typical *B. pestis* were found.

HISTOPATHOLOGY OF THE STOMACH AND INTESTINE

A catarrhal condition of the mucosa of the stomach and intestine is almost constant in bubonic plague. The hæmorrhages which have been spoken of as of such frequent occurrence in the gastric and intestinal mucosa are, as a rule, very superficial.

Near the tips of the papillæ or villi in the areolar tissue between the glands in both the stomach and intestine small hæmorrhages occur from the capillaries. These hæmorrhages may involve only the area between two glands or may include several papillæ. The vessels in the lower stratum of the mucosa and submucosa show engorgement. In simple hæmorrhages no other change is seen.

In those areas in the stomach that have been referred to as hæmorrhagic erosions there is seen a severer change. Here the hæmorrhage involves a larger area and extends more deeply into the mucosa. However, this lesion is usually entirely restricted to the mucosa, only one of our preparations showing hæmorrhage below this, and there is always a zone of hæmorrhagic glandular tissue recognizable above the muscularis mucosæ. Necrosis and sloughing of a cup-shaped area of the mucosa occurs, and the margins of the small ulcer are formed by mucosa which appears normal with the exception that hæmorrhage has occurred into it. A large number of polymorphonuclear leucocytes has been noted in the hæmorrhagic tissue. Necrosis of the cells of the upper layer of the mucosa of the stomach and intestine has been stated by many authors to be very common in plague. In my work the changes in these cells have been interpreted as a simple post-mortem digestion.

Pest bacilli are demonstrable in the hæmorrhagic areas, but I have never encountered them in very large numbers. Although these hæmorrhages have been interpreted by many, with whom I do not disagree, as due to bacillary emboli, we have failed to demonstrate such emboli in our sections. In the cases cited above as showing abscesses of the intestinal mucosa, the lesions show sharply circumscribed areas of necrosis in the mucosa and submucosa, with the presence of polymorphonuclear leucocytes, and engorgement of the vessels in the neighborhood. Numerous bacilli are seen in the necrotic areas.

Many authors on the subject of plague refer to the occurrence of swelling and hyperæmia of the intestinal lymphoid tissue, which may be readily visible to the naked eye. No such case has been seen in my series, although microscopic sections from the intestines which pass through the follicles often show marked engorgement of these structures.

PERITONEUM

The most frequent change found in the peritoneum is the presence of hæmorrhages. They occur either in the peritoneum itself or in the subperitoneal tissues. They are most abundant in those cases of femoral buboes which have marked primary

buboes of the second order in the iliac and lumbar glands, in which cases massive retroperitoneal hæmorrhages may be found about the iliac and lumbar glands, ureters, and kidneys, on one or both sides. Discrete punctate hæmorrhages may also be found scattered over the parietal and visceral surfaces. These hæmorrhages are in some cases especially abundant in the mesentery and on the inferior surface of the diaphragm.

Reference has already been made to the extensive hæmorrhages which may be found in the serosa of the sigmoid flexure of the colon when it lies in apposition to the peritoneum covering primary buboes of the second order in the iliac glands.

An acute fibrinous peritonitis may also occur in the form of a delicate fibrinous membrane on the peritoneum overlying severely affected glands, or on the serosa of the sigmoid flexure in those cases which present the conglomerate hæmorrhages, to which reference has just been made.

CARDIOVASCULAR SYSTEM

PERICARDIUM

Practically the only lesion found in the pericardium consists of hæmorrhages, and these are more frequent in the pericardium than in any other serous membrane. They were noted in 44 of our cases. They occur much more frequently over the visceral (epicardial) than over the parietal surface, and more often over the right side of the heart than over the left. They seem to be most frequent and most numerous about the auriculo-ventricular groove on the right side posteriorly. These may be very few in number and small in size, or they may be very numerous and widely scattered over the entire heart. A slight excess of fluid in the pericardium was noted in 2 cases, but the fluid is always clear, never being turbid nor sanguineous (Plate III).

HEART

The myocardium is almost always the seat of a parenchymatous or fatty degeneration, and in some cases a Zenker's degeneration was seen, similar to that which was frequently noted in the skeletal muscles. Small hæmorrhages in the myocardium were noted in a few cases. Dilatation of the right side of the heart is a not infrequent finding, and probably is an agonal event such as occurs in other acute infections.

The endocardium in a few cases shows small hæmorrhages, but in no case has any acute inflammatory manifestation been seen, nor has any reference to such an event been found in the literature of plague.

Chronic inflammatory lesions of the endocardium and myocardium have been found in a few cases, but these were indubitably due to preëxisting disease.

The vessels show no macroscopic lesions attributable to the plague infection other than the presence of hæmorrhages from them which are due to microscopic lesions, which will be described with the histology. The presence of hæmorrhagic infiltration of the walls of the larger vessels in the immediate vicinity of the primary bubo is very frequent and has been especially noted by the Austrian Commission, who conclude that this may form a portal for the direct passage of the bacilli from the primary bubo to the blood stream in the veins.

HISTOPATHOLOGY OF THE HEART

The myocardium constantly shows evidence of acute parenchymatous degeneration, with a marked swelling of the fibers. Small hæmorrhages beneath the epicardium have been noted as of very frequent occurrence. Congestion of the smaller vessels between the muscle bundles is frequently a prominent feature, and it is not unusual to see small hæmorrhages separating the muscle fibers. Cellular exudate from the vessels does not occur. Œdema may be marked and widespread, causing wide separation of the muscle fibers.

The muscle fibers often show marked vacuolation, but seldom fatty change. Some of the muscle fibers become opaque, and the striation, especially the cross striation, becomes very indistinct. Transverse clefts and even complete fragmentation of the fibers is often seen. This homogeneity of the fibers, with the breaking up into small fragments, is apparently a Zenker's hyaline degeneration of the muscle fibers. Such a degeneration was frequently noted in the macroscopic examination. Bacilli may be found within the vessels and are particularly numerous in the vicinity of the hæmorrhages.

NERVOUS SYSTEM

Meningitis in plague has been reported by the Austrian [33] and German [34] plague commissions and by Calmette and Selembini [35]. Two noteworthy cases have occurred in my series.

The first case (2431) was a 19-year-old female Filipino, who had been ill for two weeks. The anatomic diagnosis was bubonic plague; acute suppurative lymphadenitis (primary bubo), right axillary; acute suppu-

[33] Über die Beulenpest in Bombay (1898), II B, 287.
[34] *Arb. a. d. kais. Gesundheitsamte* (1899), 16, 101.
[35] *Ann. Inst. Pasteur* (1899), 13, 865.

rative ependymitis; acute leptomeningitis; acute parenchymatous degeneration of kidneys, liver, and myocardium; chronic fibrous pleurisy, left; pregnancy, sixth month. The description of the brain is as follows: The cerebral leptomeninges contain a slight excess of fluid which appears slightly turbid. On opening the right lateral ventricle, it is found to contain a considerable amount of yellowish gray pus. The choroid plexus is large and soft. The left choroid is smaller, but also surrounded by grayish exudate. The fourth ventricle is apparently free. The brain substance is pale and shows nothing abnormal.

In smears from the spleen, glands, and pus from the axilla no bacteria were found. Smears from the pus of the ventricle were loaded with plump bacilli, which varied greatly in size but did not show altogether typical bipolar staining. Agar cultures from the spleen showed a few colonies which proved to be plague. Agar cultures from the ventricles showed an abundant growth of plague bacilli; a guinea pig inoculated cutaneously with this culture died in five days with typical lesions of plague.

The second case (3247) was an 18-year-old male Filipino, who was said to have been ill for one month. This case had cervical retropharyngeal buboes, bronchopneumonia, acute suppurative meningitis, acute parenchymatous degeneration of the viscera, acute splenitis, and trichuriasis.

The meninges over the hemispheres are dry, and the convolutions are flattened. At the base of the brain, about the circle of Willis and ventral to the pons, is found considerable greenish yellow pus. The lateral ventricles contain a turbid, slightly greenish fluid, mixed with a thick, stringy, greenish yellow pus, which is adherent to the floor of the ventricles, especially at the region of the choroid plexus, which is completely enveloped in the exudate. The floors of the ventricles are thickly covered by this shaggy mass. The third and fourth ventricles contain a slightly turbid fluid.

Bacillus pestis was recovered in cultures from the exudate from the ventricles, and a guinea pig vaccinated with the culture died with typical plague lesions.

Aside from these cases of frank meningitis, the meningeal vessels in almost all cases examined showed a congestion, and in some cases the brain substance showed some œdema. No other lesions were found.

URINARY SYSTEM

The kidneys in plague invariably show evidence of an acute parenchymatous degeneration, the renal parenchyma being swollen and opaque, with relatively broad and bulging cortex. The pyramids are sometimes paler, with bluish red peripheries, and sometimes they are intensely red. In some cases the swelling, opacity, grayness, and friability of the cortex is so marked as to justify the macroscopic diagnosis of necrosis of the kidney. Hæmorrhages in the capsule, in the mucosa of the calices and pyramids, and in the renal parenchyma are frequent. The renal capsule may be studded with punctate hæmorrhages. In the pelvic mucosa the hæmorrhages may be punctate or massive so

as to fill the pelvis with blood.   The fat and areolar tissue about the pelvis of the kidney may also be the seat of extensive hæmorrhage, especially on the side on which extensive retroperitoneal hæmorrhage occurs about primary buboes of the second order in the lumbar glands.   The hæmorrhages occurring in the renal parenchyma may be numerous and punctate on the outer surface and in the cortex.   These for the most part occur in the glomeruli, constituting, along with the degeneration, a true hæmorrhagic glomerulonephritis.   However, such a condition occurs in a relatively small proportion of the cases.

The glomeruli in some cases appear pale, lusterless, and elevated.   Focal areas of necrosis, not larger than 2 millimeters in diameter, were seen twice (1969, 2989).   In both cases there were other metastatic plague foci in the viscera.

The ureters show no marked lesion other than the presence of hæmorrhages in and about them.   Punctate hæmorrhages on the mucosa occur.   Frequently the ureter on the side on which the femoral bubo occurs is completely surrounded by, and embedded in, hæmorrhagic retroperitoneal tissue, and this hæmorrhagic condition may not only be about the ureter, but may extend through its walls so as to be visible on the mucous surface at localized portions.

The urinary bladder may similarly show punctate hæmorrhages on its mucous surface; no other change has been encountered.

HISTOPATHOLOGY OF THE KIDNEYS

The morphologic changes occurring in the kidney are important and frequently characteristic.   They consist essentially of a degeneration of the tubular epithelium and an intracapillary glomerulonephritis.   Changes in the vessels also occur as well as some œdema and hæmorrhages, but cellular reaction in the form of evidence of regeneration or exudation is minimal or lacking.

The epithelium of the primary convoluted tubules undergoes a marked granular degeneration with swelling of the cells.   Frequently hyaline masses appear in the cells and the latter become broken up.   Necrosis of these cells with their desquamation into the lumen frequently occurs.   Very seldom are other cellular elements found in the tubules, except occasionally red blood cells.   A granular material is practically always present in the distended tubules.

Congestion of the vessels is frequently marked between the tubules, both in the cortex and in the pyramids.   Small hæm-

orrhages also occur in all parts of the kidney, beneath the capsule, beneath the pelvic mucosa, and in the cortex and pyramids. Œdema may widely separate the tubules, and it is not infrequent to find fine threads of fibrin in the intertubular vessels. This fibrin is attached to the walls of the vessels, and the endothelium to which it is attached may show no change; in other cases the endothelial cells are decidedly enlarged.

Glomerular changes may also be marked, and these form the most characteristic change in the kidneys in plague. However, marked glomerular changes are not present in all cases, as in some the changes in the tubular epithelium, as above described, may be predominant. Degeneration and vacuolation of the epithelium covering the tufts may be present. As a rule the changes in the epithelium lining Bowman's capsule are slight. The capsular space frequently contains a granular reticulated material, similar to that found in the tubules. Occasionally red blood cells are also found here as well as desquamated epithelial cells. Much more rarely are leucocytes found in the capsular space. Congestion of the tuft capillaries is very frequent. In 41 per cent of my cases fibrin thrombi were found in the tuft capillaries. This fibrin may be in the form of fine threads, which are adherent to the walls of some of the tuft capillaries, or may completely fill some or all of the capillaries of some or all of the tufts in a section. The larger thrombi can be readily recognized with the hæmotoxylin and eosin stain, but the smaller threads may require a selective stain, such as Weigert's fibrin stain, for their detection. The endothelium lining the capillaries does not show evidence of marked change in those cases with thrombosis.

The bacilli occur in relatively small numbers in the kidney as compared with the bubo or spleen. They may be found in the vessels of the kidney, as in all parts of the body. They are, at least, not always demonstrable in association with the vascular thrombi.

GENERATIVE ORGANS

These organs are not frequently the seat of marked change. Hæmorrhages have been encountered in the mucosa of the uterus, in the peritoneum covering it, in the ovaries, in the testes and epididymes, and along the course of the vas deferens.

In the uterus of one of our cases was a 6-month fœtus, and another uterus was enlarged, with adherent placenta and dilated cervix, the patient having aborted a fœtus of about the eighth month the day before death.

Scheube [30] cites a case of intrauterine infection with plague that was reported. by Leumann.

In my case (2431) of which the uterus contained a 6-month fœtus no pest bacilli were demonstrable in sections of the placenta or umbilical cord.

HISTOPATHOLOGY OF THE TESTES

In two cases hæmorrhages in the testes were noted macroscopically. These appear histologically as diffuse intertubular hæmorrhages, which widely separate the tubules from one another. Much of the blood has undergone hæmolysis. The cells of the tubules themselves show no recognizable alteration.

PANCREAS

No recognizable macroscopic lesion has been seen in the pancreas aside from the occurrence of occasional small hæmorrhages in its capsule and in the interstitial tissue and some congestion of the organ.

HISTOPATHOLOGY OF THE PANCREAS

No marked morphologic change has been found in the pancreas in any of the cases. In two cases large vessels within the pancreas showed thrombosis, the vessels being filled with leucocytes and a small amount of fibrin with numerous bacilli. No associated change in the surrounding pancreatic tissue was recognizable.

ADRENALS

The adrenals in plague are as a rule swollen and moist and often red. Hæmorrhages occur about the capsule of the organs, in the cortex, and in the medulla. These are as a rule small and punctate, but more diffuse hæmorrhages have been seen within the medulla.

HISTOPATHOLOGY OF THE ADRENALS

The lesions in the adrenals are not a prominent feature in bubonic plague, but there are a variety of lesions which not infrequently occur.

A degeneration of the cells especially in the zona fasciculata may be present, in which the cytoplasm of the cells appears to dissolve away, leaving only a spongelike cytoplasmic reticulum surrounding the nucleus. This may involve nearly all of the cortex, or may be restricted to a few cells. In a few cases small areas were found in which the nuclei also disappeared, constituting areas of focal necrosis. There was never seen any evidence of exudation of cells into these areas.

[30] Krankheiten der warmen Länder. Gustav Fischer, Jena (1910), 267.

Œdema of the gland is also a very common feature, and this is most noticeable between the vessels and columns of cells in the zona fasciculata. This may cause a considerable widening of the spaces between these columns of cells. Congestion of the cortical vessels is exceedingly common, and in a few cases the medullary vessels were engorged. No cellular exudate, however, appears to take place, although fine fibrin threads were found in the cortical vessels in 23 per cent of 26 adrenals examined with this point in view.

Small hæmorrhages may occur in the gland—more frequently in the cortex than in the medulla. These were found in 32 per cent of 26 adrenals that were carefully examined. Small hæmorrhages in the medulla occurred in 2 cases only.

The changes then that were found in the adrenals consisted of degeneration, œdema, congestion, hæmorrhage, focal necrosis, and fibrin thrombosis.

ASSOCIATED LESIONS

Osler's seemingly paradoxical statement that persons rarely die of the disease with which they suffer does not apply to plague. Neither is plague a disease which has any predilection for those who have had their resistance to infection lowered by chronic disease. These statements probably apply with a greater degree of truth to plague than to any other known acute epidemic disease. The virulence of the plague toxin and the rapidity of multiplication of the plague bacillus are such that they do not require a ground already prepared in order that they may exert their harmful effects to the fullest extent. Scheube [37] places the highest age incidence of plague between the ages of 25 and 30 years. Reference to Table I shows that the highest age incidence in my series was between the ages of 15 and 40 years; in other words, individuals in the prime of life are most frequently attacked. This undoubtedly accounts for the relatively small number of cases of plague that show at autopsy evidence of associated lesions.

In my series associated lesions were minimal in number. Chronic adhesive pleurisy heads the list with 20 cases. Evidence of tuberculosis (or other evidence of tuberculosis) was found in 5 cases. Chronic cardiac or renal disease, or both, was found in 5 cases only. Of uterine conditions 1 case had a myoma, 1 was pregnant, and 1 had recently aborted. Status lymphaticus was found to be present in 5 cases (1894, 2084, 2086, 2124, 2378), aged respectively 19, 14, 37, 15, and 16 years. No other associated lesions were found.

[37] Krankheiten der warmen Länder. Gustav Fischer, Jena (1910), 267.

ILLUSTRATIONS

PLATE I

Metastatic pulmonary lesions in bubonic plague seen through the pleura. Case 1969. See page 270. (Drawing by T. S. Espinosa after Kaiserling preservation.)

PLATE II

Metastatic pulmonary lesions in bubonic plague. Cut surface of same lung as in Plate I. (Drawing by T. S. Espinosa after Kaiserling preservation.)

PLATE III

Heart, showing epicardial hæmorrhages in bubonic plague. (Drawing by T. S. Espinosa after Kaiserling preservation.)

PLATE IV

Stomach, showing hæmorrhages and hæmorrhagic erosions in bubonic plague. (Drawing by J. Castro after Kaiserling fixation.)

PLATE V

(Drawn by T. S. Espinosa after Kaiserling preservation. Natural size.)

FIG. 1. Femoral bubo, showing involvement of several glands with hæmorrhage and necrosis of interglandular tissue. Case 1952.
2. Axillary bubo. Case 2048.

PLATE I. METASTATIC PULMONARY LESIONS IN BUBONIC PLAGUE SEEN THROUGH
THE PLEURA.
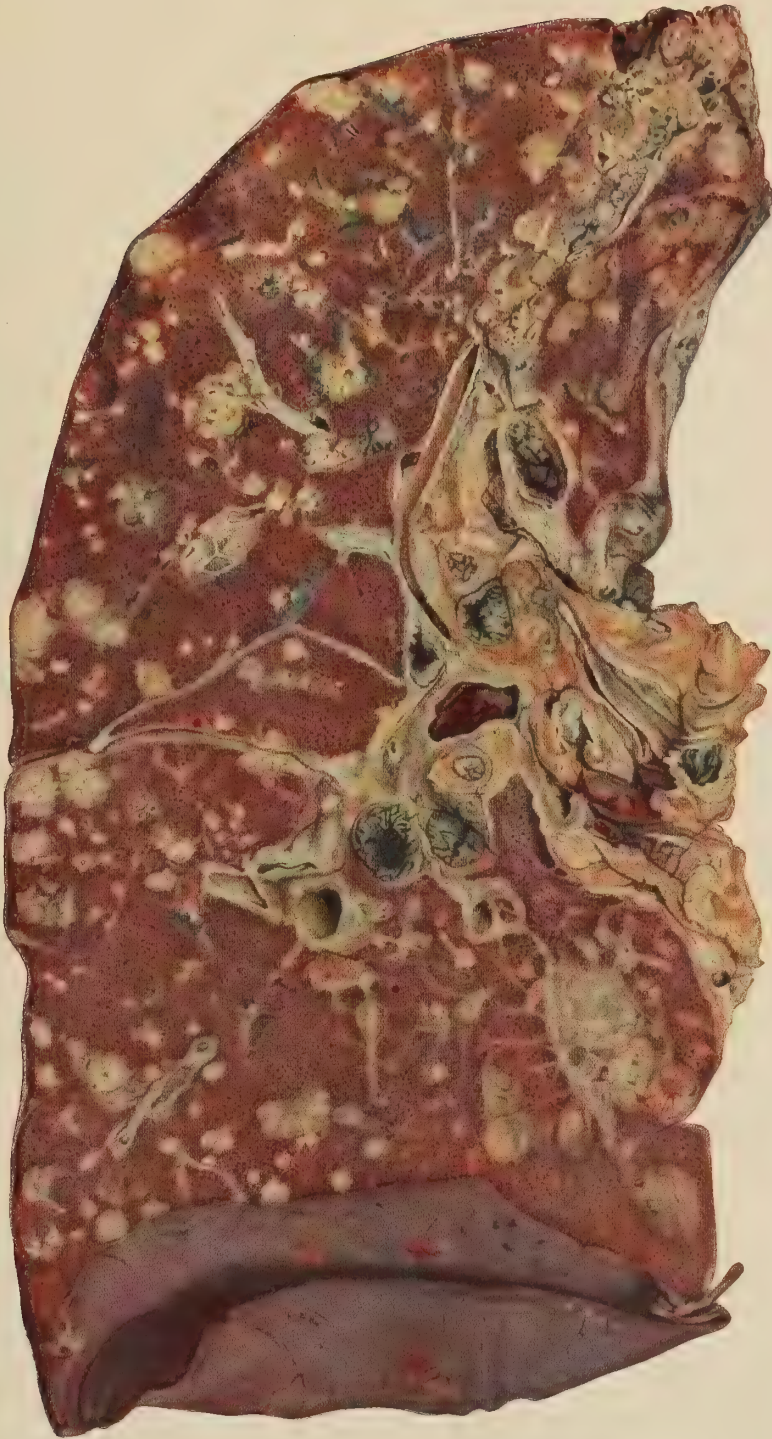
PLATE II. METASTATIC PULMONARY LESIONS IN BUBONIC PLAGUE.

PLATE III. HEART, SHOWING EPICARDIAL HÆMORRHAGES IN BUBONIC PLAGUE.
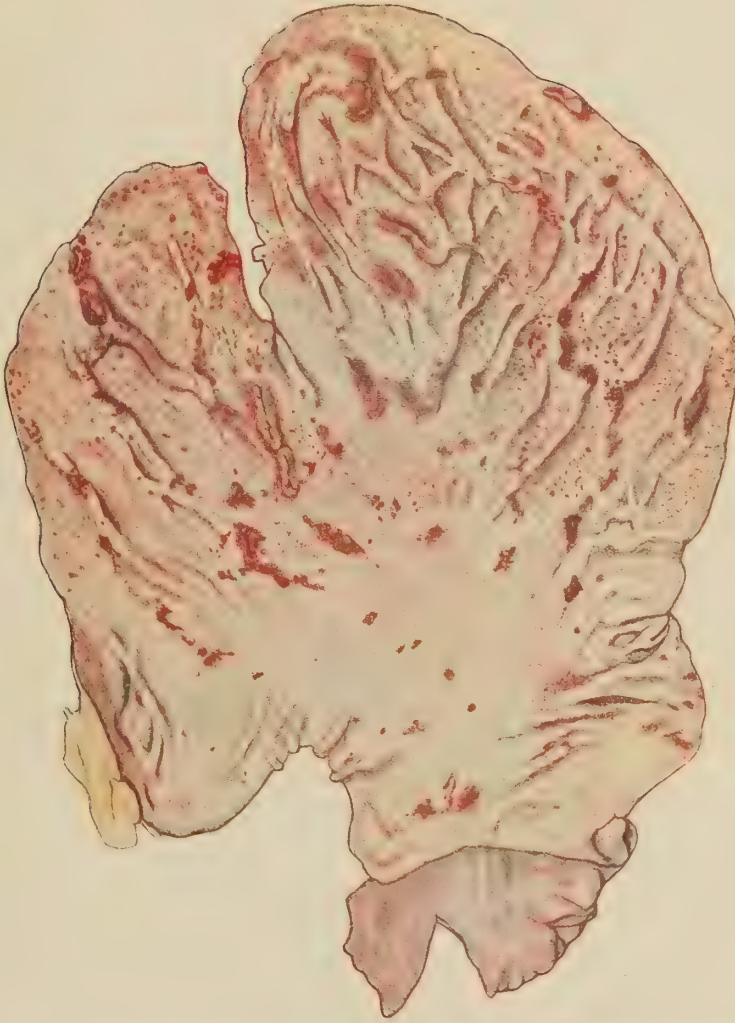
PLATE IV. STOMACH, SHOWING HÆMORRHAGES AND HÆMORRHAGIC EROSIONS IN
BUBONIC PLAGUE.

Fig. 1. Femoral bubo, showing involvement of several glands with hæmorrhage and necrosis of interglandular tissue.

Fig. 2. Axillary bubo.

PLATE V.

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I. THE RÔLE PLAYED BY THE INSECTS OF THE DIPTEROUS FAMILY PHORIDÆ IN RELATION TO THE SPREAD OF BACTERIAL INFECTIONS. II. EXPERIMENTS ON APHIOCHÆTA FERRUGINEA BRUNETTI WITH THE CHOLERA VIBRIO [1]

By DAVID N. ROBERG

(*From the Laboratory of Medical Entomology, College of Medicine and Surgery, University of the Philippines*)

It is interesting to note that so many years elapsed after the discovery of bacteriology before flies as agents in the transmission of disease were given serious attention. As early as 1886 Spillmann and Haushalter[1] isolated the tubercle bacillus, and Tizzoni and Cattani[2] isolated the cholera vibrio from flies caught in the vicinity where human cases occurred. Earlier still, in 1862, before the days of bacteriology, Budd[3] observed that flies were instrumental in the spread of malignant pustule. World-wide attention was first attracted to flies as agents in the transmission of disease when the extensive outbreak of typhoid fever occurred in the concentration camps of the American Army during the Spanish-American War, when Reed, Vaughan, and Shakespeare[4] reported the origin of the outbreak as due to flies.

There is a long list of contributors on the subject of flies, most notable of whom are those who wrote first on the development and life history of flies [Lowne,[5] Griffith,[6] Newstead,[7] Graham-Smith,[8] and Howard[9]].

Numerous contributions have been made on the carriage of infection in the alimentary tract of flies. Chief of these are

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the articles by Grassi,(10) Maddox,(11) Alessi,(12) Celli,(13) Uffelmann,(14) Sawtchenke,(15) Yersin,(16) Firth and Horrocks,(17) Manning,(18) Hayward,(19) Lord,(20) Chantemesse,(21) and Buchanan.(22)

Instances are recorded in which flies have been caught in the neighborhood of human cases infected with virulent organisms, and the interior of the flies or their dejecta have shown the presence of pathogenic bacteria. In this manner wild flies have been found to harbor cholera vibrios by Tizzoni and Cattani,(2) Simmonds,(23) and Tsuzuki;(24) typhoid bacilli by Hamilton,(25) Faichnie,(26) Bertarelli,(27) and Cochrane;(28) tubercle bacilli by Spillmann and Haushalter,(1) Hofmann,(29) Lord,(20) Hayward,(19) Cobb,(30) and Buchanan;(22) anthrax by Cao;(31) and plague bacilli by Yersin(16) and Hunter.(32)

Articles too numerous to mention here have been written on the transmission by flies of typhoid fever, tuberculosis, cholera, dysentery, infantile diarrhœa, anthrax, smallpox, ophthalmia, oriental sores, yaws, and parasitic diseases.

Of the numerous articles on the transmission of disease by flies the Diptera usually referred to are of the larger species. Heading the list is *Musca domestica,* then *Calliphora vomitoria, Lucilia cæsar, Sarcophaga carnaria,* and others.

In determining the species of most importance in relation to the spread of disease, Howard(33) examined a great number of flies in regard to their breeding places and found 36 species which bred in human fæces. Of these, especially 6 were found in dining rooms and kitchens, and thus constitute particularly a menace in the spread of disease. These species were represented by *Musca domestica, Drosophila ampelophila, Homalomyia canicularis, H. brevis, Stomoxys calcitrans, Phora femorata,* and *Sarcophaga tibialis.*

In subsequent bulletins issued by Howard no other mention is made of Phoridæ as a menace in the carriage of bacteria and spread of infection. The Phoridæ, then, are only casually mentioned in the literature, and no experimental work has been done on the family in relation to the carriage of disease.

As so much of the work done on flies is of recent date, and as it is only within a few years that their life history, habits, and their relation to disease are becoming understood, not much attention has been given this important family of Diptera.

It is the object of this paper to ascertain, by a series of experiments on *Aphiochæta ferruginea* Brunetti, whether the results obtained would indicate this fly to be instrumental in the carriage

of disease, and whether it can be considered as important as the more commonly noted *Musca domestica, Stomoxys calcitrans, Lucilia, Sarcophaga, Calliphora,* and other of the larger species which have received so much attention in the literature.

DESCRIPTION OF THE FAMILY PHORIDÆ

The family Phoridæ contains, according to Kertész, (34) 27 genera and 221 species. Malloch, (35) in reviewing the Phoridæ in the United States National Museum, enumerates 79 species under the genus *Aphiochæta.*

According to Malloch's description the insects of this family are for the most part very small, black or yellow flies, which on account of their minuteness are easily overlooked. Of the life history of this group there is very little known. Strange to say it is over a century since the first species was described, and even now but little is known of its habits. Complete life history records of this family are rare, and the list of those so recorded is very meager.

Those species that have been reared have, for the most part, been bred upon fungi and dead or decaying vegetable or animal matter. Some have been reared from snails, and a few from the bodies of bees, ants, or beetles. Whether or not some of these are true parasites is a matter of conjecture. They may be found throughout the year, especially within houses.

Malloch lists 57 species of the Phoridæ whose habits are partially known. As some of these species named may be instrumental in the carriage of disease, because of their breeding places, and as they are few in number, the names are here given.

Recovered from exhumed human bodies are: *Trupheoneura opaca* Meigen and *Conicera atra* Meigen. From a buried human corpse, *Phora aterrima* Fabricius. The following 5 species were recovered from carrion: *Trupheoneura trinervis* Becker, *T. perennis* Meigen, *Dohrniphora abdominalis* Fallen, *Chætoneurophora calignosa* Meigen, and *C. ora curvinervis* Becker.

The remainder of the 57 species occur on dead snails, bees, caterpillars, and other insects, rotting vegetable matter, leaves, or plants, or as commensals with other insects.

Brues, (36) who revised and monographed the North American Phoridæ, gives the following description of the family and of the genus *Aphiochæta.*

Although a considerable number of Phoridæ have been described during the past few years, our knowledge concerning the extent of the family is still very meager. The forms occurring in

Europe and North America are comparatively well known, but those of less accessible regions have not been so thoroughly collected as most other families of Diptera. The generic relations of the family seem to be very similar in the most widely separated geographical regions.

<div align="center">FAMILY CHARACTERS</div>

Phoridæ are probably the most readily recognized family among all the Diptera. Apart from the wingless forms they possess such a peculiar and constant type of wing venation that they may be readily recognized at a glance. The important family characters are: Small or minute species with a hunched-back appearance. Head small, hemispherical or rather flattened; front broad in both sexes, usually bearing 3 or 4 transverse rows of bristles; face very short, concave. Oral opening large, the palpi well developed and projecting, usually with strong bristles. Eyes never very large, often much reduced in wingless forms; ocelli always present in the winged forms. Antennæ of peculiar form; the third joint large and concealing the others, spheroid or pointed, with a long, nearly bare subdorsal or apical arista. Thorax usually large and arched above, the scutellum rarely absent. Abdomen short, more or less slender and narrowed behind in the male; in the female more oval in shape and pointed at the tip. Genitalia of the male usually prominent, often large. Those of the female small and projecting. In a few genera the ovipositor is hard, chitinized, and projecting. Legs well developed; coxæ stout, the anterior ones very long and free at the base; femora stout; the posterior pair often swollen or flattened; tibia generally with a few strong bristles, sometimes bare. Wings usually large, but sometimes very small or entirely absent in the female. When present, they have a series of two heavy veins anteriorly which reach only halfway to the apex of the wing, and three or four much lighter ones which run obliquely across the disc of the wing.

<div align="center">GENERIC CHARACTERS</div>

The most important characters for the definition of genera are to be found in the presence or absence of strong macrochætæ on the tibia, the position of the frontal bristles, and the venation of the wings, especially with regard to the branching of the third vein. The reduction of the wings and the remarkable elongation of the ovipositor are important for the recognition of some genera.

GENUS APHIOCHÆTA BRUES (37)

Characters.—Head usually rather small, the front usually about quadrate, with 4 rows of bristles; anterior row consisting of 2 or 4 closely placed, proclinate; three upper rows of 4 each normally, although the median pair of the lower row is sometimes absent. Eyes moderately large, ocelli on a tubercle. Frontal impressed groove generally present. Antennæ oval, with a dorsal arista. Palpi scarcely ever strongly enlarged, usually with strong bristles. Proboscis usually small, but sometimes enlarged or heavily chitinized. Cheeks each with a pair of macrochætæ. Thorax with a single pair of dorsocentral bristles. Abdomen oval or long and tapering in the female, much slenderer in the male, with projecting hypopygium. Ovipositor retractile, soft, and fleshy. Legs without preapical bristles on the tibia, although the four posterior ones are sometimes finely setulose. Spurs present on four hind legs. Wings ample, the third vein forked at the apex, mediastinal vein present.

APHIOCHÆTA FERRUGINEA BRUNETTI

Aphiochæta ferruginea was described by Brunetti.(38) Although the flies were accidentally reared from a dead lizard in 1907, the description was not published until 1912. Brunetti describes this species at length as he found it to correspond with some specimens of flies at the Indian Museum sent there by Doctor Crombie, obtained from the fæces of a European in Rangoon suffering with myiasis. In the fæces were passed eggs and maggots as well as adult flies.

According to Brunetti *A. ferruginea* has a wide distribution throughout the tropical regions and some adjacent parts of the temperate zone, also.

Brunetti's description is as follows:

♂ ♀. India, Ceylon. Long. 2¼–3 mm.

Head.—Brownish yellow, yellowish, often more or less tinged with grey, sometimes entirely greyish. Eyes black, microscopically pubescent, posterior orbit with a row of short black bristles; two strong macrochaetae below the lower angle of each eye, and a vertical row of short bristles on the cheeks. Antennae pale yellowish, varying to brownish yellow, arista long and microscopically pubescent. Palpi brownish yellow or yellowish, with several shorter bristles on outer side, a few hairs on inner side and five or six separated strong spines towards the tip. Frons generally concolorous but sometimes with brownish irregular marks in the middle on a yellowish ground colour, the vertical impressed line sometimes very distinct. The frons is furnished with four rows of four macrochaetae each. The upper or vertical row are reclinate, equidistant,

the outer ones at the upper angles of the eyes, the median pair on the inner side of the two upper ocelli, and slightly below the vertical margin. *Behind* the vertical margin is a bristle on each side, almost immediately contiguous to the outer bristle of the vertical row. These post-vertical bristles are convergent. The second row is just below the lower ocellus, and consists of four equidistant bristles, the median pair fractionally but perceptibly higher on the frons than the outer ones. The bristles in this row are generally reclinate but sometimes are almost at right angles to the frons. The 3rd and 4th rows are so composed as to almost make a single semicircular row of eight. They probably, however, represent two rows of four each, those forming the 3rd row placed thus: the outer pair near the eyes as usual (and immediately under the outer ones of the 2nd row but rather more distant vertically from them than these latter are from the vertical or 1st row); the inner bristles rather closer than usual to the outer ones, leaving a wide space of the frons between the inner pair, which latter are much lower on the frons than the outer pair. Of this row, the outer ones are always reclinate, but the median ones are often more nearly horizontal.

The 4th row is placed wholly on the middle of the frons in a short semicircle, the two median ones the lowest, and the outer ones still proximad of the median pair of the 3rd row. Of this 4th row, the outer ones are more or less horizontal, the median pair always very distinctly proclinate.

In some specimens the outer pair of the 3rd row are rather further removed from the median pair, and in this case these latter alone may be considered as forming the 3rd row, and the median pair, with the outer two of my fourth row, as forming a row of four representing the 4th row. There would then be a pair of additional proclinate median bristles below this fourth row. The previous interpretation of their arrangement is apparently the truer one.

Thorax.—Generally bright ferruginous or brownish yellow, varying in shades and with or without an admixture of grey; occasionally with two faint greyish dorsal lines. Some strong bristles of unequal length and number laterally from the shoulders (where there is always one strong humeral bristle) to the posterior corners, there being generally four towards the hinder border of the dorsum. Some stronger ones in front of the wings; three small ones close together near the fore coxæ, a fan-shaped row of six or more small but distinct ones at the extreme base of the costa, and a similar row behind the base of the wing. Sides of the thorax rather lighter; scutellum concolorous, with four strong bristles, the outer pair usually the larger.

The whole surface of the thoracic and scutellar dorsum is covered with minute stiff bristles.

Abdomen.—Dark ferruginous or brownish yellow, practically bare and *very variable.* Normally brownish yellow, the posterior half (or thereabouts) of each segment black or blackish, but this colour sometimes occupies the greater part or whole of one or more segments; in some specimens a clearer space, more or less oval in shape, remains in the centre of the segments; or the abdomen may be wholly black or blackish, with or without pale edges to the segments, this character itself being present or absent irrespective of other coloration.

Sometimes the abdomen is black or dark reddish ferruginous with the centre part generally pale, forming a light wide irregular dorsal stripe. Belly generally brownish yellow or some kindred shade.

Genitalia in ♂ dark, not prominent, bluntly conical, with a few hairs; in ♀ the ovipositor is subcylindrical, pale yellowish, with two stiff hairs at tip.

Legs.—Pale dirty yellow, minutely pubescent. Coxae with some strong black bristles at tip. Femora without distinct bristles, the hind pair are often paler in colour, and are generally blackish towards the tips, often only very slightly so. Tibiae with a distinct row of short bristles on outer side, the four posterior tibiae with two terminal spurs, the hind pair having a close row of very minute hairs on the outer side, adjacent to the row of bristles. Tarsi slightly blackish.

Wings.—Pale yellowish, a little iridescent, costal border shortly bristly with two divaricating rows as far as the tip of the 3rd longitudinal vein, which, shortly forked, reaches about the middle of the wing, the 1st longitudinal ending just beyond the middle of the costal cell. Of the light veins, all of which attain the wing-margin, the 1st takes a single, distinct curve upward, the 2nd and 3rd are moderately bisinuate (sometimes much less so), the 4th is nearly straight. Halteres pale yellow. * * *

Note.—This species is very variable in colour, ranging from bright ferruginous to grey, the proportion of black on the abdomen totally altering the general appearance of the insect. The species, however, once well understood, is tolerably easy to recognize.

Concerning the life history of *Aphiochæta ferruginea,* Brunetti's [39] notes showed that it required a period of from twelve to fifteen days for the first generation to emerge. Pupation occurred from five to eight days after the eggs were laid. Emergence of the imagines took place seven days after pupation. As the eggs were not noted at the time they were laid, there is a variance of three days in the process.

The first generation laid eggs when they were five or six days old. Three days after pupation a few imagines emerged. Due to the scarcity of food the majority of the second generation emerged from sixteen to twenty days after the eggs were laid.

The specimens of flies which were secured and employed in the experiments outlined in this paper were compared with the identified specimens in the Bureau of Science collection at Manila by Prof. Charles S. Banks, and were found to correspond with *Aphiochæta ferruginea* collected by him. There is, also, a correspondence with the description given by Brunetti.

Before going into detail concerning the object of the experiments in this paper, a review of the literature will be gone into concerning the phenomenon of the larvæ of dipterous insects transferring pathogenic bacteria from the medium upon which

they feed into the emerging imagines. The question of flies becoming infected by means of the pathogenic bacteria taken up by the larvæ from which they develop is of importance from a public hygienic standpoint.

Concerning this question there have been but five investigators who have reported their results. The first of these, Cao,(31) in 1906, made observations upon the larvæ of the following diptera: *Musca domestica, Calliphora vomitoria, Lucilia cæsar,* and *Sarcophaga carnaria,* they being feeders on putrefied meat or carrion.

Feeding these larvæ on putrefied carrion, and then securing cultures from their intestinal flora, he demonstrated that death resulted in guinea pigs in from three to five days after being injected by these cultures. From the guinea pigs could be isolated a virulent strain of *Bacillus coli,* two or three varieties of typhoidlike organisms, and less frequently a pseudomalignant œdema bacillus, *Staphylococcus aureus,* an anthraxlike bacillus, and a very virulent *Proteus fluorescens.*

The intestinal flora of the larvæ were like those which are present during putrefaction, being *Proteus vulgaris, P. mirabilis, P. zenkeri, Bacillus subtilis, B. radiciformis,* an anthraxlike bacillus, *B. fluorescens liquefaciens, B. fluorescens nonliquefaciens,* cocci, typhoidlike and colilike bacilli, and less frequently the sarcinæ, *Bacillus prodigiosus,* the oïdia, and blastomycetes.

As a result of his investigations Cao came to the following conclusions:

The bacterial intestinal flora of larvæ is like the bacteria which occur in the putrefying meat upon which they feed.

The intestinal flora consists of the *Proteus* group, the *subtilis* group, the fluorescent group, coli and typhoidlike organisms, and cocci.

The bacterial flora is alike in the flesh of cold- or warm-blooded animals and alike in the different species of flies.

The intestinal flora of larvæ acquired during feeding upon putrefied meat shows a greater virulence than those existing in the meat.

The germs in putrefying dead animals are derived from the air, from their intestinal contents, from the blood of those dying of septicæmia, and from flies as they deposit their eggs.

The interior of the eggs of flies is sterile, but the shell contains many bacteria.

Bacteria introduced into the intestinal tract of flies are passed in the fæces, living and virulent, and contaminate the surface of the eggs as they are laid.

The pathogenic bacteria occurring in meat also occur in the intestine of larvæ feeding thereon.

Pathogenic bacteria occurring in the pupa are transmitted to the imago which in turn disseminates infection in its fæces.

In 1909 Faichnie,(26) following Cao's suggestions, used *Bacillus typhosus*.　His procedure was as follows:

Into a box of earth containing three ounces of fæces, infected with typhoid bacilli, 30 flies were let loose and covered with a wire gauze.　In a day or two all these flies died, but fourteen days later 1 fly emerged.　On the fifteenth day 12 more emerged. On this day the box was replaced by an earthenware dish washed in bichloride of mercury, and water and sugar were introduced as food and covered.

The 1-day-old fly was transfixed with a red-hot needle, flamed and washed in 1 cubic centimeter of sterile salt solution, part of which was inoculated into McConkey's broth, which remained unchanged for forty-eight hours.　This same fly, when crushed in sterile salt solution and a drop plated, showed the presence of typhoid bacilli.　Four other 1-day-old flies gave identical results.

Two flies 6 days old and two 9 days old gave the same results.

Two flies 13 days old showed fæces infected with *B. typhosus*.

One fly 16 days old showed typhoid-contaminated fæces; when crushed it showed the same contamination.

Faichnie concludes that of 13 flies bred from a typhoid stool at least 6 contained the bacillus in the intestines and that a 16-day-old fly contained bacilli both in the intestines and fæces.

The results described by Faichnie are to be questioned, because there is no evidence in his paper that the larvæ fed on the infective material were separated from the pupæ or newly emerged adult flies.　There is no evidence to exclude the possibility that the emerging adults were not reinfected by feeding on the typhoid fæces.　(The species of flies used are not mentioned.)

In considering later the work done by Ledingham, it will appear remarkable that the typhoid bacilli in the fæces remained capable of infecting the flies for a period of twenty days.　In Ledingham's paper it will be further pointed out that typhoid

bacilli are weak in competing with the intestinal bacterial flora of larvæ and that they are soon outgrown by the more vigorous organisms mentioned by Cao.

A. W. Bacot,(40) in 1911, employed *B. pyocyaneus* in order to determine its persistence from the pupæ to the imagines of *Musca domestica*. His technique and results are as follows:

A vessel containing sterile sand, mixed with baked milk, cooked rice, gristle of meat, and other food, had added to it the eggs of *M. domestica*. Several cultures of *B. pyocyaneus* were added to this, and the growing larvæ were fed thereon.

Some of the larvæ were secured; their surfaces were disinfected by washing them in 5 per cent lysol for five minutes. These surface-sterilized larvæ when crushed and inoculated into broth showed a growth of *B. pyocyaneus*.

Flies just as they emerged were secured, and their surfaces, likewise, were sterilized in lysol. These flies had no opportunity to infect themselves by feeding upon *B. pyocyaneus*. When crushed and inoculated into broth, they showed a growth of *B. pyocyaneus*.

Ledingham confirmed these results by securing the pupæ of flies, and in place of sterilizing the surface with lysol, held them between the fingers, searing the anal extremity with a red-hot knife-point, passing a blunt pipette into the intestines and withdrawing the contents which were then mixed with salt solution and sprayed on plates. This modified technique was more satisfactory, because pupal disinfection by lysol is difficult, owing to the fact that, when the pupæ are shaken in broth or sterile salt solution, bacteria escape from their interiors.

Ledingham,(41) in 1911, having confirmed Bacot's work, performed a series of experiments to ascertain whether or not an organism like *B. typhosus* could adapt itself to conditions prevailing in the larval and pupal interior in competition with organisms of a more hardy nature. His work includes the following:

In the first series eggs of *M. domestica* were placed in a sterile dish, containing sterile sand mixed with sterilized food in the form of mashed potatoes, meat, and turnips. This mash was repeatedly drenched with broth cultures of typhoid bacilli.

While the larvæ were growing and feeding, the mash developed a strong ammoniacal odor.

As this mash was so liberally drenched with cultures of typhoid bacilli, plates of McConkey's medium were inoculated with the mash to ascertain whether or not *B. typhosus* would be the

predominating organism. On examination none of the plates showed the presence of *B. typhosus*. Constantly found was a typhoidlike organism, provisionally called *Bacillus "A."* *Bacillus prodigiosus* was also found. The strong ammoniacal odor was liberated by *Bacillus "A."*

Pupæ were taken from the mash; their exterior was sterilized, and then crushed and inoculated on plates of McConkey's medium. These plates showed profuse growths of *Bacillus "A"* and *B. prodigiosus,* but *B. typhosus* could not be recovered.

Similar examination of the larvæ before pupation resulted, also, in the isolation of *Bacillus "A,"* but not of *B. typhosus.*

A newly emerged fly was chloroformed, and its exterior was sterilized. The intestines were removed aseptically, mashed, and inoculated on to plates. There was an absence of *B. typhosus.* *Bacillus "A"* was recovered.

In the second series cultures were made from the shells of eggs from which the larvæ had just hatched. These cultures showed growths of the following organisms: *Bacillus "A," B. proteus vulgaris, B. prodigiosus,* and a streptococcus.

Larvæ which had fed upon a sterile mash were secured, and their surfaces were sterilized. When mashed and inoculated upon plates, they showed growths of *Bacillus "A," B. prodigiosus,* and a lactose-fermenting organism provisionally called *Bacillus "B"* No. 3.

In the third series the eggs were first sterilized in lysol and then placed upon sterile agar and fed with sterile human blood to which were added typhoid cultures. The larvæ secured from this mixture, when their surfaces were sterilized, gave pure cultures of *B. typhosus* when crushed and inoculated upon plates.

Other larvæ were removed from this agar-human blood and typhoid mixture, placed upon sterile sand, and allowed to pupate. These pupæ showed upon examination that they contained pure cultures of *B. typhosus* when crushed.

Ledingham's conclusions are summarized as follows:

Although typhoid bacilli were liberally supplied to the larvæ, *B. typhosus* could not be isolated from the pupæ or imagines, until recourse was had first to the disinfection of the eggs.

When unsterilized eggs were used there appeared constantly a typhoidlike bacillus. This typhoidlike bacillus, called *Bacillus "B,"* thoroughly adapts itself to the conditions in the interior of the larvæ, pupæ, and imagines.

There was, also, evidence that *B. typhosus,* when isolated from the interior of larvæ fed upon typhoid bacilli under ideal

conditions, was not in a state of proliferation but that of dying out.

The presence of other bacteria in the mash made it difficult for *B. typhosus* to exist in competition with them.

Graham-Smith,(42) in 1911, made observations on the ways in which artificially infected blowflies (Sarcophagidæ) carry and distribute pathogenic and other bacteria.

Larvæ of the blowfly were fed on meat artificially infected with the spores of anthrax bacilli. Flies emerging from these larvæ were infected with anthrax bacilli and remained infective for fifteen days.

Larvæ were fed on meat infected with the respective nonspore-bearing organisms, *B. typhosus*, *B. enteritidis*, *B. prodigiosus*, *Vibrio choleræ*, and a pink-colonied coccus. Examination of the emerging flies did not reveal the presence of any of these organisms.

This indicates that nonspore-bearing organisms do not survive sufficiently long to be found in the emerging blowflies.

Later, in 1911, Graham-Smith(43) made more extensive observations along these lines and employed the larvæ of *Calliphora erythrocephala*, *Lucilia cæsar*, and *Musca domestica*.

SERIES 1

Graham-Smith, failing to infect the emerging flies from larvæ fed on artificially infected food, secured larvæ of *C. erythrocephala* and *L. cæsar* and fed them upon the flesh of guinea pigs which had died from infection with *B. enteritidis* and *B. anthracis*. Several emerging flies when examined did not show the presence of spore-free anthrax bacilli or of *B. enteritidis*.

SERIES 2

Here the females of *M. domestica* were allowed to lay their eggs upon a mixture of boiled meat, potatoes, and rice. Individual groups of larvæ were secured and placed in separate sterile sand boxes, and each was fed respectively on *B. prodigiosus*, Morgan's bacillus, *B. enteritidis*, *B. anthracis*, and a pink-colonied coccus. The intestinal contents of the emerging flies when examined showed negative results in the following: *B. prodigiosus*, *B. enteritidis*, and the coccus. Examination of the flies emerging from larvæ fed on Morgan's bacillus gave a positive result. Of those emerging from larvæ fed on anthrax bacilli, positive results occurred in 78 per cent of the cases.

These results indicate that nonspore-bearing organisms, such as *B. prodigiosus*, *B. enteritidis*, and certain cocci, cannot sur-

vive long enough in the larval intestine to appear in the imagines.

Morgan's bacillus, which is often a commensal in the intestine of larvæ, constantly persists in the intestine of the imagines, along with other nonlactose-fermenting organisms. The spores of *B. anthracis,* also, persist.

SERIES 3

To ascertain whether different substances on which larvæ were fed in any way favor the persistence of different bacteria, separate boxes were prepared and the following sterilized substances were placed therein: namely, in the first cooked meat and rice, in the second human fæces, and in the third box unsterilized human fæces. Eggs and larvæ grown on sterilized food were placed in these boxes and infected separately with *B. typhosus, B. enteritidis,* Morgan's bacillus, and *B. prodigiosus;* one box was uninfected as a control.

When the flies emerged the intestinal bacteria were examined. Growths on plates of McConkey's medium showed several nonlactose-fermenting colonies, none of which proved to be *B. typhosus.* Morgan's bacillus could be isolated from flies from the different foods.

Different foodstuffs, then, apparently exercise no influence on the persistence of bacteria in the intestines of newly emerged flies.

As previously stated, observations and attention have been particularly centered upon the larger and easily visible species of flies. This is especially true in Manila, where it is stated that a scarcity exists in flies. The scarcity of flies in Manila applies only to the larger species and not to the minute members of the family Phoridæ, which are abundant. No doubt as a result of their minuteness they have been overlooked.

These minute Phoridæ, then, having biological features similar to the common large species of Diptera, should be regarded as a menace in the spread of bacterial infections.

The members of this group lay their eggs and breed in putrefying material, and if human fæces are available, they are the more preferable.

Even though sanitary regulations be severe in regard to the disposal of human fæces, and suitable so-called fly-proof sanitary pails be recommended or furnished by the Bureau of Health, these minute flies nevertheless gain ready access to the fæces. Should the fæces be contaminated with pathogenic bacteria, as they so frequently are from cholera carriers or active cholera cases, and from infections where pathogenic organisms are dis-

charged with the fæces, it is easy to conceive how these small flies, when feeding thereon, can disseminate infections by the carriage of bacteria either upon the surface of their bodies or contained within their intestinal tracts. As they are omnivorous feeders when they enter homes, they may either contaminate food upon which they feed or become a source of danger by being ingested with the food.

Special emphasis is laid upon their minute size, as they can pass through the ordinary so-called fly-proof screen with great ease. Entrance may be gained through very small apertures or cracks into food-containing vessels, and the fly imprisoned and killed therein. Pails or chambers which are used for the deposit of human fæces, although safe against the common house fly, are by no means safe against the ingress of these very minute insects.

Aphiochæta ferruginea Brunetti is a very common species in the Philippines. As this species has been reported as producing intestinal myiasis in man, a field of speculation is opened of grave importance, should this prove to be of commoner occurrence than has been reported. Cases of intestinal myiasis may readily be overlooked, by regarding the larvæ or eggs as being deposited in the fæces after they have been passed. Adult flies may be passed per rectum and fly away and not be seen.

Should myiasis occur in a case of cholera, and adult flies be discharged, dissemination would occur by means of these cholera-drenched flies.

Before too much significance is attached to the Phoridæ as porters or carriers of bacterial infection, certain facts must be established by experimentation regarding them.

In the experiments outlined in this paper *Aphiochæta ferruginea* was chosen for the following reasons: (1) Its great frequency as a breeder in human fæces. (2) When once determined it can readily be recognized. (3) It is a trifle larger than the other members of the family and can be handled with less difficulty. (4) No experimental work has been performed on this species since it was discovered. (5) Facts ascertained concerning this species will open a field of investigation appertaining to the entire family, of which very little is known.

The object in view is to establish the following facts:

1. Whether or not cholera vibrios are harbored during the chrysalis stage.

2. Is infection transmitted from the chrysalis stage to the imago?

3. Do adult flies, when fed on cholera vibrios, harbor these

organisms on their surfaces or in their intestinal tracts; and for what length of time do they remain infective?

4. Which media are preferably chosen by these flies for breeding?

5. Biological features concerning the development of the fly.

6. Ability of the flies to pass through small apertures.

TECHNIQUE EMPLOYED IN EXPERIMENTS AND A CONSIDERATION OF
THE FINDINGS ELICITED

In the four series of experiments outlined here are recorded the results of positive findings. For the purpose of brevity and clearness the positive findings are described separately from the negative.

The obstacles encountered and the technique developed in the course of the experiments are best described before outlining the series of experiments.

In series I, concerning the biological features of *Aphiochæta*, little difficulty was met with. The minute Phoridæ were readily trapped, as the vessels used as traps were placed in a screened room which excluded the larger species of flies. As traps were covered with cloth gauze, only minute flies could gain entrance therein. When a considerable number of flies had been caught, they were kept imprisoned until they had laid their eggs.

These imprisoned flies, when seen through the glass wall of the vessel containing them, for the most part presented abdomens of a brownish yellow ground color, with transverse blackish stripes. The thorax was characteristically hunched. These characteristically colored and hunched-back flies were uniform in size and averaged 2.5 millimeters in length. A number of these flies were removed from the traps by covering the traps with a glass funnel, and as the flies passed up the stem of the funnel they were caught in a glass Erlenmeyer flask inverted over the upright funnel stem. When killed and mounted on needles, they were found to be *Aphiochæta ferruginea*, as previously stated and described.

These flies, having a characteristic appearance and color, could be readily recognized in the vessels containing them; therefore, as they could be seen as the predominating flies contained in the traps, the eggs when laid and hatched out would give origin to a new generation of flies, of which *Aphiochæta ferruginea* would predominate greatly over others which might be present and not recognized on account of their similarity to *Aphiochæta*.

Among these brownish yellow flies could be seen a few which

were entirely black and smaller in size. These were regarded by Banks as males of *Aphiochæta*. Brunetti also states in his description of *Aphiochæta ferruginea* that there is a variance in color.

When a medium of sand was employed, containing food in the form of sugar, bread, human fæces, and bouillon, the eggs developed into a great number of flies, the great majority of which were the brownish yellow *Aphiochæta*. There were, also, present fewer numbers of those having a blackish appearance.

To identify the flies, a considerable number were secured immediately upon emergence and studied with a magnifying glass. Most of those examined were found to be female *Aphiochæta;* others, the small and darker males. As it was impossible to examine all the flies which emerged by this method, it was possible that other allied species were present whose similarity might cause them to be confused with *Aphiochæta*.

The period of development could be definitely ascertained, because when the flies emerged they were secured and identified as *Aphiochæta*.

In series II and III considerable difficulty was encountered where experiments were performed to ascertain whether larvæ when growing in a medium infected with cholera vibrios contain these organisms in their intestines, and whether a transference of vibrios occurs from the larvæ, through the pupæ and into the emerging imagines.

Repeated attempts were made to keep larvæ alive on a medium containing a pure growth of cholera vibrios. Larvæ could exist for only a day or two on growths of cholera vibrios on agar-agar and Dieudonné's medium. On agar-slant growths of vibrios to which sand was added larvæ could subsist for four or five days.

Larvæ could be kept alive for nine days on an agar-slant growth of cholera vibrios containing sterile sand liberally soaked with bouillon. In this case flies were allowed to enter the test tube containing the vibrio growth and sand by inserting a bent glass tube into the test tube and connecting it with a flask containing flies. The flies traveled from the flask into the tube, where they were imprisoned sufficiently long to allow them to lay their eggs, after which they were allowed to pass along a bent glass tube into another flask. The larvæ, although living for nine days, did not attain their full size nor did they pupate.

From this tube 6-day-old larvæ were removed with a sterile platinum needle and examined to see whether their intestines contained cholera vibrios. To remove the surface vibrios, the

larvæ were washed for five minutes in a 5 per cent solution of lysol. The larvæ were then washed three times in sterile salt solution and placed in Dunham's peptone solution, after which they were crushed with a sterile glass rod. This material was incubated for twenty-four hours and streaked on Dieudonné's medium. Growths of cholera vibrios were looked for.

Three attempts to find vibrios in the intestines of 6-day-old larvæ failed by this method. It was believed that in killing the surface vibrios with 5 per cent lysol sufficient liquid was ingested by the larvæ to kill the vibrios contained in the gut. To preclude any possibility of lysol entering the larval interior, the larvæ were sealed by applying a red-hot platinum point to the anal extremities, thus searing and closing the openings. Larvæ treated in this manner and washed in lysol also did not reveal the presence of any cholera vibrios. Failing to find vibrios within the larvæ, the agar-slant growth was examined for vibrios and found to contain none, but did possess other contaminating organisms.

To keep larvæ alive on a medium containing cholera vibrios until emergence of the adults occurred, it was found necessary to obtain a medium as free as possible from putrefying material; to use larvæ of an age which would soon pupate; and repeatedly to drench the sterile sand with 24-hour-old cholera broth cultures. This method is outlined in series III. In this series the cholera vibrios were identified by smears stained with Sterling's gentian violet, by the characteristic growth on Dieudonné's medium, by typical motility in hanging-drop preparations, and by agglutination of the vibrios in hanging drops by the addition of cholera-immune serum.

In series IV experiments were performed to determine whether cholera vibrios are harbored in the intestinal tract of flies which have been fed upon media containing vibrios, and the length of time they remain infective; if vibrios adhere to the surface of the body; and how long they remain infective.

In performing these experiments it was necessary to use extreme care in handling the minute flies which were infected with cholera vibrios to prevent their escape. Under ordinary circumstances they can be caught with a forceps as they attempt to escape when the lid is raised from the vessel containing them.

Before cholera-infected flies were handled, various methods were tried in order to find a means whereby it would be impossible for them to escape. The most satisfactory means was the employment of a bent glass tube which served as a passage-

way for the flies from one flask to another, or from test tube to test tube.

The procedure in this series of experiments consisted of the following: The bent glass tube was inserted between the cotton stopper and the neck of the flask containing a number of uninfected flies, and the other end of the bent tube was inserted in a like manner into a test tube containing an agar-slant growth of cholera vibrios. When a number of flies had passed from the flask into the test tube, the bent connecting tube was removed. The flies were allowed to remain in the test tube until they had fed upon the cholera growth and were then passed into a clean flask by means of the bent connecting glass tube. On some occasions it required about two hours for the flies to pass from one vessel to another. Attempts were made to hurry the flies by darkening or heating one of the vessels; this, however, seemed to have little effect.

Infected flies were removed from time to time from the flask and passed into a tube of Dunham's peptone solution by means of the bent tube. The flies were washed in Dunham's peptone solution to ascertain whether or not any vibrios were present on the surface of the fly. This washing was incubated for twenty-four hours and streaked on Dieudonné's medium. A second Dunham's peptone solution was employed for the same fly which was crushed with a sterile glass rod. This was incubated for twenty-four hours and then streaked on Dieudonné's medium. The growth on the Dieudonné plate was studied morphologically by means of smears stained with Sterling's gentian violet. Hanging-drop preparations were observed for the characteristic vibrio motility, and the final identification was made by securing an agglutination by the addition of cholera-immune serum to the hanging drop.

In determining the presence of vibrios on the fly surface or within its gut, after various intervals of time had elapsed since the feeding of the flies on vibrios, it was necessary to examine a great number of flies bacteriologically. In series IV are recorded only the positive findings.

In a considerable number of instances the infected flies were killed in handling them or they died in the flask containing them, as they were given no food nor drink. Furthermore, in many of the flies examined negative findings occurred. This may have been due to insufficient enrichment of the vibrios by not making enough subinoculations into Dunham's peptone solution. It was found that when only one fly was employed four or five transfers into Dunham's peptone were necessary to enrich the vibrios

present, in order to isolate them on Dieudonné's medium.   When four or five flies were employed, it was necessary to enrich the vibrios present in only one or two changes of Dunham's peptone solution.

OUTLINE OF EXPERIMENTS

SERIES I. BIOLOGICAL FEATURES

On the evening of March 29 four traps were set to catch flies, with the object of securing *Apiochæta ferruginea* and to breed them after their eggs had been deposited upon the substances contained in the traps.

Three of these traps consisted of wide-necked 8-ounce bottles, containing a perforated stopper in which was placed the stem of a glass funnel.   Over the orifice of the funnel was stretched a single layer of cloth gauze, having 20 squares to the linear inch. The fourth trap consisted of a 6-inch beaker, over which was stretched a layer of the same gauze.

Into each of the three bottles were placed, respectively, the following food substances for breeding the flies: agar-agar, a mixture of human fæces and melted agar, and a dead snail.   In the beaker was placed a mixture of human fæces and melted agar.

On the following morning (March 30) these traps were set at 5-foot intervals in a room, carefully screened with wire netting (having 16 squares to the linear inch), which prevented larger flies from entering the room.

In the vicinity of the beaker, which smelt strongly of fæces, there began soon to appear a few small flies which could scarcely be seen at a distance of 3.5 meters.   During the course of the day great numbers of these hovered about the beaker; some were seen to alight upon the surface of the gauze, readily pass through into the beaker, and settle upon the fæces-agar mixture, which they greedily devoured.

The bottle trap which, also, contained a fæces-agar mixture, smelt less strongly, and fewer flies were seen hovering about it. Some of them passed through the gauze and into the bottle.

The bottle containing the dead snail smelt of putrefaction, but fewer flies were seen there than in the two previous traps.

The bottle containing plain agar attracted no flies.

On the morning of March 31 a considerable number of flies were still in the bottle and beaker which contained the fæces-agar mixture.   These were covered to prevent further ingress or egress of flies and kept for observation.

The bottle containing the dead snail contained only a few flies.   This was covered.

The bottle containing the agar-agar had but two flies. This was covered.

The flies from these four traps were removed and placed in separate flasks for identification. For the most part these flies averaged 2.5 millimeters; some were smaller. Specimens of the larger type were compared with members of the Phoridæ in the Bureau of Science collection, identified by Professor Banks. These flies corresponded with *Aphiochæta ferruginea* of that collection and coincided with Brunetti's description.

Before the present series of experiments was undertaken, I had exposed, on March 4, a large gauze-covered specimen jar containing fæces. In this jar hundreds of small flies had emerged. These proved to be *Aphiochæta ferruginea,* and were saved as a stock supply for future experiments.

On April 1 great numbers of small larvæ were seen crawling about the fæces-agar mixture in the bottle and beaker. Fewer were seen on the dead snail. On the plain agar they were not yet visible.

On April 6, the bottle containing the fæces-agar mixture being small and having a great number of larvæ measuring from 2 to 3 millimeters, the entire contents were placed into a large specimen jar containing sand, lumps of sugar, bread, fæces, and bouillon. Eight layers of cloth gauze (having 20 squares to the linear inch) were stretched over the orifice to prevent new flies from entering.

Upon transferring the contents it was seen that a number of the larvæ were pupating. Examination of larvæ which had not yet pupated showed on their blunt extremities, on the inferior portion thereof, two small hooks not unlike those described by Brunetti as appearing like two small walrus tusks. The body is whitish yellow, and the dark intestinal contents are visible through the body wall.

On April 8 a newly emerged fly made its appearance. On the following day (April 9) flies appeared in great numbers. On examination the great majority were found to be *Aphiochæta ferruginea.*

It is interesting to note that some of the flies escaped from the jar, passing through eight layers of gauze tightly stretched over the surface.

The emerged flies were allowed to remain in the jar. On April 14 deposits of small eggs were seen on the sides of the jar. These are whitish with a tinge of yellow, and measure 0.2 by 0.5 millimeter. The extremities are rather blunt, and there is a slight curve in the long axis.

Summary of results.—Specimens of *Aphiochæta ferruginea* readily pass through a wire screen having 16 squares to the linear inch. It is possible for them to pass through 8 layers of tightly stretched gauze having 20 squares to the linear inch.

The time elapsed from the laying of the eggs to the emergence of the adult is from nine to ten days. Eggs are laid by the flies five days after emergence. Pupation of the larvæ takes place five or six days after they are hatched.

By indoor-breeding experiments *Aphiochæta ferruginea* are indicated as the commonest of the small flies which breed in fæces.

SERIES II

To ascertain whether larvæ when growing in a medium infected with cholera vibrios contain these organisms in their intestinal tracts, the following experiments were performed:

a. On April 1 eggs were removed from the beaker trap (series I) and placed on a cholera culture growing on a Petri plate of Dieudonné's medium. This plate was placed in a large glass-covered stender dish to await the hatching of the larvæ.

b. On the same day a similar plate culture of cholera was placed in a large gauze-covered stender dish containing fæces to attract flies, which in turn would deposit their eggs upon the medium. As soon as the dish was exposed, a small fly was seen to enter through the gauze and immediately leave the dish again. As it was possible for the flies to leave the dish after entering it and disseminate infection, this method of securing eggs was abolished.

The eggs placed on Dieudonné's medium in *a* hatched out in twenty-four hours. The larvæ lived for twenty-four hours and then died from a lack of nourishment.

c. On April 2 three larvæ were secured from the bottle of fæces-agar mixture (series I) and placed on the surface of a cholera growth in a test tube of slanted agar. These larvæ when removed from the bottle showed their intestines to contain a dark substance which could be seen through the larval wall. After feeding for twenty-four hours the intestinal contents became colorless. Two days later the larvæ died.

SERIES III

In *a, b,* and *c,* of series II it was impossible to keep larvæ alive longer than two days on a pure culture of cholera vibrios, as there was a scarcity of nutriment in the agar and Dieudonné's medium.

The following steps were undertaken to keep larvæ alive in a medium containing cholera until emergence of the imagines should take place.

As was noted in series I, a fly emerged on April 8 in the large jar. On April 10 there were, in addition to the emerging flies, a considerable number of large and well-advanced larvæ. As these larvæ were due soon to emerge as imagines, they were selected because they would have only a short time to be cultivated. They measured from 3 to 4 millimeters in length.

a. Thirty of these larvæ were removed from the large jar and placed in a sterile glass-covered stender dish containing sterile sand. The sand and the larvæ were drenched with a broth culture of cholera vibrios. A fresh hen's egg was opened aseptically and the albumen transferred with a sterile pipette into the stender dish as food for the larvæ.

b. On the following day (April 11) no active larvæ could be seen, as several of them had pupated.

One of these pupæ was removed with a sterile platinum loop and placed in 5 per cent lysol for five minutes, in order to kill the cholera vibrios adhering to its surface. After three washings in sterile salt solution to remove the lysol, a final washing was made in a tube containing Dunham's peptone solution. This tube was incubated for twenty-four hours and then streaked on Dieudonné's medium. Examination of the plate showed the absence of cholera vibrios. There was present, however, a growth consisting of a coccus and a spore-bearing bacillus.

The lysol-sterilized and washed pupa was placed in a tube containing Dunham's peptone solution and macerated. A portion of the contents of this tube after being incubated for twenty-four hours was streaked on Dieudonné's medium. Plate examination revealed the presence of cholera vibrios. There was, also, a growth containing a coccus and a spore-bearing bacillus.

c. Three new pupæ were secured and subjected to the same steps as in *b.* The results proved identical.

d. A portion of the sand was inoculated into Dunham's peptone and later streaked upon Dieudonné's medium. Examination showed typical colonies of cholera vibrios. There were, also, present colonies consisting of cocci and variously formed bacilli, many of which contained spores.

e. On April 14 some actively moving larvæ were noted. These had not yet pupated.

One of these larvæ was removed, and the extremities were

seared with a red-hot platinum point. It was then placed in
5 per cent lysol for five minutes, washed three times in sterile
salt solution, and the final washing in Dunham's peptone was
placed in a tube and incubated for twenty-four hours. This
tube was perfectly clear and did not show any bacterial growth.

The lysol-disinfected larva, when crushed in Dunham's pep-
tone, incubated for twenty-four hours, and plated on Dieudon-
né's medium, did not reveal the presence of cholera vibrios.

f. Sand inoculated into Dunham's peptone, incubated and
plated on Dieudonné's medium, did not reveal the presence of
cholera vibrios. Several variously formed bacilli and cocci
were present.

g. As the results of *e* and *f* indicate that the vibrios had
been outgrown, not only in the sand but also in the intestinal
tract of the larvæ, it was necessary again to replenish the cholera
vibrios by the addition of 24-hour-old broth cultures to the
medium in which the larvæ were developing.

The larvæ and pupæ were removed and placed in a new
sterile stender dish containing sterile sand. This was again
drenched with broth cultures of cholera vibrios.

h. On April 16, as no flies had emerged, another transfer was
made to a new sterile dish and again drenched with vibrios.

i. On April 18, as no flies had emerged, they were again
transferred and redrenched with vibrios.

j. On April 20 one fly emerged. As I was not present at the
time of emergence, it was not tested for vibrios contained in
its intestines. Another transfer was made into a sterile dish
and again drenched with vibrios.

k. On the morning of April 21 six or seven flies had emerged.
Four of the pupæ which were soon to emerge into flies were
removed and placed on a sterile moist filter paper and covered
with a beaker. During the day these pupæ were constantly
watched and late in the afternoon one fly emerged. Immediately
upon emergence the fly was chloroformed to prevent its escape
or possible reinfection from cholera vibrios on the filter paper.

This fly was placed in 5 per cent lysol for five minutes and
washed three times in sterile salt solution. The final washing
was in Dunham's peptone, which was incubated for twenty-four
hours and then plated on Dieudonné's medium. Examination
of the plate showed no cholera vibrios.

The lysol-disinfected and washed fly was crushed in Dunham's
peptone solution, incubated for twenty-four hours, and streaked
on Dieudonné's medium. Examination of this plate revealed
the presence of cholera vibrios.

Larvæ when fed on a medium containing cholera vibrios harbor these organisms in their intestinal tracts only as long as cholera vibrios are present in the medium and sufficiently plentiful in numbers not to be outgrown by other organisms.

There is a transference of vibrios from the larvæ to the pupæ and from the pupæ to the imagines. This, however, is only possible when the larvæ and the pupæ are constantly changed to a sterile medium and drenched with 24-hour-old broth cultures of cholera vibrios. If this is not done, the vibrios are outgrown by the bacteria which are commensals in the intestines of the larvæ and by the bacteria which are associated with putrefaction of the medium.

In *b* and *c* the failure of complete disinfection of the pupal surface was probably due to bacteria derived from the pupal intestine as it was shaken in Dunham's peptone solution.

The delay in the emergence of the flies is accounted for by the scarcity of nutriment present.

SERIES IV

Series 4 was undertaken to determine whether cholera vibrios are harbored in the intestinal tract of adult flies which have been fed upon media containing the vibrios, and the length of time they remain infective; and to determine if vibrios adhere to the surface of the body, and how long they remain infective. The following experiments were performed:

a. On April 1 a Petri dish of Dieudonné's medium, on which there was a good growth of cholera vibrios, was placed in a large stender dish containing human fæces and covered with a layer of gauze. Three flies were seen to alight upon the gauze and enter the dish, after which it was securely covered and the flies allowed to remain therein for sixteen hours. The flies had a choice of both fæces and vibrios upon which to feed. At the expiration of sixteen hours the flies were removed and placed in separate flasks.

b. When ten hours had elapsed since the feeding on vibrios, one fly was secured and placed in Dunham's peptone and washed for five minutes. This tube, containing the bacteria washed from the fly's surface, was incubated for twenty-four hours. As the growth was slight it was successively inoculated into three changes of Dunham's peptone to enrich the vibrios which might be present. It was then inoculated on Dieudonné's medium and examined. The plate contained pure cultures of cholera vibrios.

The washed fly was placed in Dunham's peptone and crushed. This was incubated for twenty-four hours and reinoculated into three successive changes of Dunham's peptone for enrichment and plated on Dieudonné's medium. The plate did not reveal the presence of cholera vibrios.

c. When twenty-six hours had elapsed after the vibrio feeding, the second fly was secured and washed in Dunham's peptone. This was incubated twenty-four hours, enriched in peptone three times, and inoculated on Dieudonné's medium. The plate when examined did not reveal the presence of cholera vibrios.

The washed fly was placed in Dunham's peptone, crushed, incubated, enriched three times in peptone, and inoculated on Dieudonné's medium. The plate showed pure cultures of cholera vibrios.

d. When thirty-four hours had elapsed since the vibrio feeding, the third fly was taken and subjected to the processes recorded in *b* and *c*. Cholera vibrios could neither be found in the surface washing nor in the crushed fly.

e. A number of flies were taken from the stock jar and allowed to feed upon an agar-slant culture of cholera vibrios. After being fed, they were placed in a flask. When twenty-two hours had elapsed since they were fed on vibrios, three of them were secured and placed in Dunham's peptone to wash off the surface bacteria. This was incubated for twenty-four hours and then enriched three times in peptone and plated on Dieudonné's medium. The examined plate showed no cholera vibrios.

The washed flies were crushed in Dunham's peptone, incubated for twenty-four hours, enriched three times in peptone, and plated on Dieudonné's medium. Examination of the plate showed pure cultures of cholera vibrios.

CONCLUSIONS FROM RESULTS

Aphiochæta ferruginea, when fed on media infected with cholera vibrios, harbors the organisms on its body for ten hours and in its intestinal tract for twenty-six hours.

Failure to isolate cholera vibrios from the crushed fly in *b*, in which ten hours had elapsed since it was fed on cholera vibrios, was no doubt due to a killing of the organisms by using a too hot platinum needle in making inoculation transfers.

SUMMARY AND CONCLUSIONS

Aphiochæta ferruginea is a species of fly which breeds in human fæces, and as indicated by indoor laboratory experi-

ments, is the commonest of the small flies which breed therein. Its period of development is from nine to ten days, and eggs are laid when the fly is 5 days old. The promptness with which fæcal or putrefying material attracts these flies when kept indoors suggests that they are not scarce.

Their minute size enables them to pass through the ordinary fly-proof screens used as prophylactic means against the invasion by the common house fly and other large species of Diptera.

In these series of experiments sufficient evidence is furnished to indicate this species of Diptera as a possible porter or carrier of Asiatic cholera.

These flies may serve as agents in the dissemination of Asiatic cholera and, by analogy, other alimentary infections, such as typhoid fever, bacillary dysentery, and infantile diarrhœa; by ingested food which has been contaminated by organisms from the fly's body surface and fæces; or by ingestion of the entire fly which may have become incarcerated within the food.

Fly-proof wire-screen bell jars employed in restaurants, kitchens, and tiendas to protect food against the common house fly do not exclude the Phoridæ.

Fly-proof sanitary pails which are ordinarily used for the deposit of human excrement, although proof against the common house fly, may not be secure against invasion by the Phoridæ.

The fact that cholera vibrios may be transmitted from larvæ, through pupæ, into emerging imagines is of importance from a public hygienic standpoint only under exceptional circumstances. This is possible only if fæces heavily infected with cholera vibrios should be deposited among larvæ which are due to emerge as adults in a day or two.

Chantemesse,[21] in studying the spread of cholera in Europe, lays special emphasis upon the common house fly as a carrier of cholera vibrios by contaminating food with vibrios contained on their feet and in their fæces. He demonstrated that flies harbor vibrios in the tubes of their feet and in their fæces for seventeen hours.

Cholera vibrios have been isolated by Ganon[44] from flies twenty-four hours after they had been fed on infective material. Graham-Smith recovered them in the fæces and on the legs after thirty hours, and in the crop and gut after two days.

In the Philippine Islands, where there are many questions unsolved in the epidemiology of Asiatic cholera, the insects of the dipterous family Phoridæ are worthy of serious consideration.

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MISCELLANEOUS NOTES [1] AND COMMENTS ON BERIBERI [2]

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It is a matter of common belief among physicians who have had practical experience with beriberi that the incidence of the disease is highest, other things being equal, among those upon whose metabolic processes the greatest demands are made. Thus it has been generally observed that women during the period of the puerperium are very subject to beriberi even though the other members of the same household, other than the children of the beriberic mother, do not betray the slightest symptoms of the disease. At various times mention has been made of a high incidence of beriberi among those performing unusually hard manual labor, such as firemen on board ship.

The effect of heat and physical exercise upon the onset of polyneuritis in fowls was tested in the following experiments:

Four fowls were fed on polished rice under ordinary conditions; 4 were fed on unhusked rice (palay) under the same conditions; 4 were fed on white rice and given from ten to twenty minutes exercise twice daily in a treadmill moving at a rate of about 25 meters per minute; 4 were fed on palay and given the same exercise; 4 were fed on white rice in cages in which the air temperature was maintained at from 37° to 38° C.; 2 were fed on palay in cages kept at the same temperature; and 2 were fed on white rice and kept in cages in which the air temperature was maintained at 15°.

The results indicate that high temperatures and physical work slightly hasten the onset of the disease. They are more interesting as illustrations of the general inadequacy of a white rice diet, since birds fed on white rice succumbed under these conditions even before definite symptoms of polyneuritis had time to develop.

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[1] These notes have been collected at the close of an extended period of investigation of beriberi, although much of the experimentation is incomplete. The results and comments are, therefore, offered only as suggestions.

[2] Received for publication April 23, 1915.

TABLE I.—*Record of experiments with fowls.*

No. of fowl.	Condition.	Duration of experiment.	Weight change.	Day of death.	Symptoms and remarks.
		Days.	*Per cent.*		
1	White rice control		—26.5	Thirty-first	Typical neuritis.
2	_____do		—29.9	Twenty-ninth	Do.
3	_____do		—21.7	Twenty-second	Do.
4	_____do		—40.8	Twenty-eighth	Do.
5	Palay control	60	+ 3.0		Alive and well at end of 60 days.
6	_____do	60	— 1.2		Do.
7	_____do	60	+ 4.8		Do.
8	_____do	60	+ 1.2		Do.
9	White rice and 40 minutes exercise.		—27.0	Seventh	Apparent exhaustion after exercise. No neuritis.
10	_____do		—14.5	Fourth	Do.
11	White rice and 20 minutes exercise.		—15.3	Twentieth	Typical neuritis.
12	_____do		—20.2	Twenty-fourth	Do.
13	Palay and 40 minutes exercise.	60	— 6.0		Alive and well at end of experiment.
14	_____do	60	— 8.2		Do.
15	Palay and 20 minutes exercise.	60	— 5.6		Do.
16	_____do	60	— 5.0		Do.
17	White rice at 37°		—24.1	Fourth	Prostration without neuritis.
18	_____do		—35.2	Sixteenth	Typical neuritis.
(70) 19	_____do		—64.2	Thirty-third	Do.
(73) 20	_____do		—34.0	Tenth	Prostration and slight neuritis.
(71) 21	Palay at 37°	60	—18.0		Alive and well at end of 60 days.
(72) 22	_____do	60	— 8.8		Do.
23	White rice at 15°		—51.9	Thirtieth	Typical neuritis.
24	_____do		—21.5	Forty-fourth	Do.

That beriberi is due to an intoxication of some sort has been the basis of several theories of the etiology of the disease. Such a hypothesis has much support in the symptom complex, especially of acute beriberi. Very similar forms of neuritis are produced by alcohol, arsenic, and some other poisons and a few toxins. Since the discovery of the vitamines there has been a general tendency to ignore this evidence and accept the idea that beriberi is due to a deficiency of vitamines per se. A theory that regards the vitamines simply as constituents necessary for tissue building fails to account rationally for a number of recorded facts regarding beriberi in men and animals.

Perhaps the most cogent argument against such a supposition is that beriberi in its severest form almost invariably develops very rapidly.   Often within the space of a few days an apparently healthy and normal person reaches a condition of severe prostration.   Recoveries are likewise prompt upon the administration of vitamines in some form.   A process of starvation with respect to some essential food would be expected to produce a very gradual development of the symptoms.   It is true, of course, that chronic human beriberi does often develop in this way.   In fowls fed exclusively on polished rice gradual appearance of the symptoms is not observable in most cases, but nevertheless frequently occurs.   In a series of 150 fowls fed exclusively on polished rice 10 have survived at the end of periods of from four to eight months.   Of the 10, four showed marked lameness and spasticity of gait at the end of from thirty to forty days, which condition continued from three to ten days, after which they recovered except for a very slight stiffness of gait and an apparent dullness of sensation.   The latter condition was evidenced by a retarded response when prodded or otherwise disturbed.   It amounted to nothing more than a slight torpidity.   The remaining 6 fowls gradually developed the mild, chronic condition without passing through an acute stage.   It may be noted here that among the 150 fowls at least 3 showed an unmistakable œdema in the feet.

The development of beriberi in breast-fed infants is particularly rapid.   When completely weaned from the breast and fed artificially on fresh milk rapid improvement frequently begins. In a number of cases we have noted that a single nursing at the breast causes a prompt and more or less severe exacerbation. Such a result would not appear to be accounted for by a simple deficiency, the less so since Gibson [3] has shown that fresh milk does not possess antineuritic properties in any extraordinary degree.

In addition, Sawazaki [4] has recently reported the production of a paralytic condition in fowls by injection of the milk of beriberic women.

The existence of various very dissimilar forms of beriberi have led to a classification as wet and dry, acute and chronic. This classification, however, is very indefinite and its value may be questioned.   The only distinction that need be drawn

[3] *This Journal, Sec. B* (1913), **8**, 469.

[4] *Mitt. Med. Gesell. Tokio* (1913), **27**, No. 3, Abstract in *Zentralbl. f. Biochem. u. Biophys.* (1913), **15**, 314.

for practical purposes is that between beriberi of short and long duration. Nevertheless the more detailed classification has been made the basis of considerable argument and speculation regarding the identity as to etiology of the various forms of the disease. Of the essential identity we feel less and less doubt in the light of further experience. Chronic beriberi in mothers and the acute form in nursing infants are almost invariably associated with each other and all types are benefited by the same treatment.[5]

These facts, namely, (1) the symptom complex, (2) the rapid development of, and recovery from, acute beriberi, (3) the properties of the milk of beriberic mothers, and (4) the apparent close association of all types of the disease, have led us to formulate a working hypothesis somewhat as follows: That in beriberi there exists a toxic substance which produces the symptoms of the disease. If produced rapidly or in great quantities this toxic substance brings about a condition similar to anaphylactic shock, resulting in acute beriberi. If gradually developed there results chronic beriberi with progressive nerve lesions. This toxic substance may be produced by a hypothetical organism or, as may perhaps seem more probable, may be the product of normal or slightly disturbed metabolism. The vitamines are then necessary antidotes for the poison and are, therefore, to be regarded as therapeutic agents rather than foods. Incidentally it may be mentioned here that the results of the chemical investigation of the vitamine, which are to be published shortly, bear out this view rather than otherwise.

Our efforts to demonstrate the presence of such a toxic substance in beriberic fowls have for the most part been unsuccessful. Nevertheless the occasional positive results obtained warrant further effort along this line as the possibilities have not been exhausted by any means. The problem is doubtless somewhat complicated by the apparent presence of some vitamines in the carcasses of pigeons.[6] The fact that alcoholic extracts of such carcasses are curative rather than poisonous is no argument against the existence of a toxic substance, as the latter would probably be destroyed or eliminated by extraction with alcohol.

Our first experiments consisted in the injection of the defibrinated or citrated blood of neuritic fowls into the veins of healthy birds. Quantities of from 5 cubic centimeters to 20

[5] Williams and Saleeby, *This Journal, Sec. B* (1915), 10, 99.

[6] Funk, *Zeitschr. f. physiol. Chem.* (1914), 89, 373.

cubic centimeters of blood were used in injections into 7 fowls without obtaining any certain positive indications of transmission of the disease. In two fowls the wings drooped noticeably for two or three days following the injections. Four fowls into which blood was injected had previously been fed for ten days on polished rice in the hope of increasing their susceptibility to the disease. Even these birds, on a continued white rice diet, failed to develop polyneuritis any earlier than controls.

A number of attempts were made to transfuse the blood of neuritic fowls into healthy ones. These were unsuccessful on account of the mechanical difficulties encountered.

The entire fresh carcasses of three birds which had died from polyneuritis were ground in a meat grinder and the fluids expressed from the flesh. In each case the entire fluid expressible from a carcass was introduced into the crop of a healthy bird. No neuritic symptoms appeared in these fowls within thirty days, during which time they were fed on unpolished rice.

The kidneys, liver, spleen, and heart of a bird which died of polyneuritis were removed and minced up together. About 5 grams of the minced tissues were fed to each of three healthy fowls, which were thereafter fed on unhusked rice. This experiment was repeated with the organs of three neuritic birds. Of the total of 9 fowls receiving the mixed minced tissues of the internal organs only one showed unmistakable signs of neuritis. This bird, nine days after ingestion of the diseased organs, developed all the typical symptoms of polyneuritis precisely as do fowls fed on white rice. The second day thereafter the bird became completely prostrated, displayed increasingly severe neck retraction and labored breathing, and died. This is the first case we have observed of the development of apparently typical polyneuritis in an animal fed exclusively on a diet supposedly rich in vitamines and known to be highly antineuritic. Several of the remaining fowls displayed more or less severe wing drop and torpidity during the second and third day, but later recovered completely.

A further series of fowls was submitted to repeated dosage with the organs of diseased fowls. Data on this series is given in Table II.

McCarrison [7] has reported the isolation of an organism in cultures from the liver, spleen, kidney, and heart of birds in which polyneuritis had been developed by feeding on white rice.

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[7] *Indian Journ. Med. Research* (1914), 2, 369.

TABLE II.—*Feeding experiment of repeated dosage with the organs of diseased fowls.*[a]

Fowl No.	January 27.[b] Weighed (Grams.)	Fed organs of fowl No. 77.	January 28. Remarks.	Fed organs of fowl No. 70.	January 31. Remarks.	February 1. Fed organs of fowls Nos. 52 and 48.	Remarks.
78	1,418	Spleen	Wing drop		Condition unchanged	Spleen No. 52	No change.
79	1,245	Kidney	do		do	Kidney No. 52	
80	916	Liver			do	Liver No. 52	Better.
81	1,005	Heart	Wing drop		do	Heart No. 52	
82	1,275			Spleen	Wing drop	Spleen No. 48	Do.
83	1,235			Liver	No visible effect	Liver No. 48	Do.
84	1,085			Heart	do	Heart No. 48	Slight wing drop.
85	1,220			Kidney	do	Kidney No. 48	Do.

Fowl No.	February 2. Remarks.	February 3. Remarks.	February 4. Weighed (Grams.)	February 6. Fed organs of fowl No. 75.	Remarks.	February 9. Fed organs of fowl No. 57.	February 12.[c] Remarks.
78	Marked wing drop	Same	1,399			Spleen	Wing drop.
79	Sick and stupid	Knees weak	1,254		Slight spasticity	Kidney	Weak knees, slightly spastic.
80	Marked wing drop	Same	910			Liver	Wing drop.
81	Severe wing drop	do	1,010			Heart	Severe wing drop.
82		do	1,210	Spleen			Wing drop.
83		do	1,210	Liver			Slight wing drop.
84	Marked wing drop	Better	1,062	Heart			Wing drop.
85	do	Knees weak	1,219	Kidney	Slight spasticity		Weak knees, slightly spastic.

[a] All birds are affected, but the kidney-fed ones are the most seriously affected.
[b] Began feeding palay.
[c] Stopped palay and began feeding polished rice.

NOTE.—Fowl 78 died March 25; fowl 79 died March 20; fowl 80 died March 22; fowl 81 died March 8; fowl 82 died March 5; fowl 83, March 1, slight wing drop, legs slightly spastic, died March 5; fowl 84, sick March 5; fowl 85, died March 19; fowl 85 died March 11.

By injection of these cultures he was able to reproduce in fowls a disease which appeared to be typical polyneuritis. He reported the transmission of the disease by this means in a large percentage of cases.

We have repeated McCarrison's experiments without being able to verify any of his essential findings.

Ten fowls with polyneuritis gallinarum were examined by us, and cultures from the heart blood, spleen, liver, and kidneys remained sterile in each case except one, and in this case we obtained a small Gram-negative bacillus with a tendency toward bipolar staining. There was no tendency to gas production in mannite, glucose, or galactose, and but slight acidity. Rabbits inoculated intravenously with 1 cubic centimeter of a suspension of a 24-hour growth on agar died in from fourteen to twenty hours. Five tenths of a cubic centimeter of a similar suspension produced no results. Eight fowls inoculated intramuscularly with 0.5 cubic centimeter of a similar suspension showed no effects, except that a slight wing drop ensued in one, probably the result of manipulation, as it was noticeable only on one side.

Cultures of the milk of 4 beriberic women were examined with negative results.

PSEUDOTYPHOID FEVER IN DELI, SUMATRA (A VARIETY OF JAPANESE KEDANI FEVER)[1]

By Wilhelm Schüffner

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THREE PLATES

INTRODUCTORY

In the course of my practice in Sumatra I have, since 1902, met with a number of cases which, though resembling enteric fever in their general clinical characters, appear from the results of bacteriological investigation to constitute a distinct disease. At the Bombay Medical Congress in 1909 I referred to these cases under the name pseudotyphoid and in collaboration with Dr. Margarethe Wachsmuth published an account of them in the same year.

Further observation during the past five years has shown that the disease is preceded by an initial lesion in the form of a small area of dermal necrosis in some part of the body; this necrosis is followed by the formation of a small ulcer and more or less pronounced enlargement of neighboring lymphatic glands. These facts, together with the occurrence of a rash, show that the disease possesses many of the features which characterize kedani or tsutsugamushi fever of Japan. Until recently this disease has been known only in Japan, but in 1908 Ashburn and Craig described an analogous disease in the Philippine Islands, and it is probable that it occurs in other countries, also.

As I have had no opportunity of observing personally the Japanese disease, it is impossible for me to compare it in detail with the disease which occurs in Sumatra. I must, therefore, content myself with an enumeration of the more outstanding points of resemblance and difference.

COMPARISON OF PSEUDOTYPHOID WITH KEDANI FEVER

SEASONAL INCIDENCE

Kedani fever appears in Japan only at certain times of the year, which are determined by the periodical floods. In Sumatra there is no such regularity; the disease is observed throughout the year. The 158 cases during 1908 were distributed as follows:

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TABLE I.—*Incidence of kedani fever in Sumatra.*

Month.	Number.	Month.	Number.
January	15	July	19
February	12	August	19
March	10	September	7
April	6	October	5
May	5	November	25
June	16	December	19

From this distribution one can say that the disease occurs most frequently during the months of June to August (54 cases) and during the months of November to January (59 cases). These two periods are not at all alike so far as meteorological conditions are concerned; the period June to August is dry, while the period November to January is the time of greatest rainfall.

MORTALITY

The second important difference is in regard to mortality. In Japan it is accepted that an average mortality of about 30 per cent occurs; according to older writers it was as high as 70 per cent. In advanced age the disease is especially dangerous. In Sumatra the mortality is only about 3 per cent, and though the estate laborers are for the most part young, this low mortality shows that despite the grave symptoms observed in its course the disease must be classed among the less dangerous maladies. Enteric fever in Deli is accompanied by a mortality of about 15 per cent; it is much more dangerous than pseudotyphoid.

TRANSMITTING AGENTS

There is, also, a difference in the transmitting agents of the disease in the two countries. In Japan a small, red mite, the larval form of an unknown *Trombidium,* is known to be the infecting agent; the true host of this mite is the field mouse, which harbors the parasite often in large numbers about the ears. It has been shown experimentally by the Japanese investigators Miyajima and Asakawa that the mite is the transmitting agent of the virus from mouse to man; they were able to infect monkeys by allowing mites to feed on these animals. Up to the present I have been unable to determine the transmitting agent of pseudotyphoid in Deli, but here, also, judging from the histories given by patients, it is a question of ticks or mites. On the estates where the disease occurs the laborers suffer greatly from the attacks of minute acarines, red in color

and so small as to be scarcely visible to the naked eye. These acarines have been examined by Professor Nuttall, who found them to be of two kinds. One is the larval form of a *Trombidium* and resembles, therefore, the kedani mite. The other one, with the long legs, is the larval form of a *Cheyletus*. It has not been possible to determine the species in either case, as the adults are unknown.

The larval *Trombidium* of Deli differs from the Japanese form as figured by Tanaka in the structure of the body and of the mouth parts; the measurements correspond to those of the small form of the kedani mite (0.15 millimeter broad and 0.25 millimeter long). The Deli mite is thick-skinned, not easy to crush, and its bite causes a violent itching after about fifteen minutes, while the bite of the Japanese variety may remain unnoticed until several days have elapsed.

In Deli we consider these acarines as suspect only, lacking direct proof of their association with the disease; their existence in large numbers in the dangerous areas and the analogies which the disease presents with kedani fever would appear to justify this suspicion. Some of my patients had been bitten by a larger acarine, which I believe may be the nymph of a species of *Hyalomma* and which I have often observed to attack man.

It can be affirmed with certainty that the Deli disease is transmitted to man by the bite of an arthropod and that it is not directly contagious. Moreover it is probable that there is a reservoir of the virus in another host as in the case of the field mouse of Japan. The development of the disease among recent immigrants in areas previously uninhabited proves this to be the case, unless one accepts the possibility that the virus may remain alive for a long time in man.

SYMPTOMATOLOGY OF PSEUDOTYPHOID FEVER OF DELI

THE DERMAL NECROSIS

In 39 per cent of cases the original point of infection is discoverable; in Europeans, in all cases. It is much easier to recognize the ulcer, often very small and after a time not very characteristic, on the healthy skin of a European than it is on that of the native, who is frequently a sufferer from other skin affections. It is for this reason that for a long time I overlooked the connection between the disease and the ulcer.

In the earliest stage that I was able to observe, the lesion showed itself as a flat vesicle, 3 to 4 millimeters in diameter,

surrounded by a dull red areola. The papule soon bursts, and beneath it there appears a small, dark area of blackish necrosed skin some 4 millimeters in diameter; five to eight days later the slough is cast off, leaving a small, round, or oval ulcer with steep edges and the floor covered with mucopus. In most cases there is slight evidence of local reaction. The ulcer is of indolent character, shows small tendency to healing, and may persist throughout the illness. The typical initial ulcer is distinguished from other forms of skin ulcerations by its clean-cut borders and from furuncles by the fact that the lesion is only superficial. Lymphangitis has not been observed, but the lymphatic glands in the neighborhood of the ulcer are enlarged and tender, sometimes markedly so; in some cases I have noted glands as large as a pigeon's egg. Such glands remain freely movable, however, as there is no inflammation of the periglandular tissue. The general lymphatic system shares in the infection, but remote from the lesion the glands are only slightly affected.

The site of the initial lesion varies, but is commonest in the regions of the groins, the armpits, and the neck. As the lesion is minute, it is frequently recognized with difficulty. In some such cases enlarged glands have been sought for and thus the bite has been located.

That the dermal affection is an essential part of the disease has been shown in a small epidemic among the Europeans on one of the estates. In May, 1909, three Europeans became ill, each showing similar symptoms which varied only in severity and duration. In all of them I found the characteristic initial lesion with its accompanying lymphadenitis. This feature I hold to be constant in the pseudotyphoid of Deli; where no such lesion has been observed, either it has already healed or has been overlooked—this I believe occurred in regard to the former series of cases which I reported. Until we learn more of the etiology of the malady, it is unwise to admit the existence of two diseases, differing as they do only in unimportant clinical details.

THE RASH

The second characteristic symptom is an eruption which appears on the second or third day of the disease and attains its full development on the sixth to eight day; it then presents itself as roseola, the raised spots varying in size from that of a hemp seed to a threepence. It closely resembles the roseola of secondary syphilis. The rash covers most of the body, being thickly placed on the flanks and less marked on the face and extremities; it persists from eight to ten days longer, then changes to a

brownish color, and slowly disappears. In some cases the eruption may be very slight, consisting only of a few reddish spots. In this rudimentary form, while it is distinctive in the European, it can very easily be missed on the dark skin of the native. To this I attribute the fact that with natives I found the rash only in 70 per cent of my cases, while with Europeans it was present in all. On the other hand, the rash may be so pronounced as to resemble the eruption of measles—in one of my cases some spots on the abdomen became hæmorrhagic, and in this case only was there desquamation.

THE TEMPERATURE CURVE

The course of the fever can best be described by saying that it corresponds in all respects to that seen in enteric fever. In severe cases the temperature attains its maximum in four or five days and so remains for some time, then gradually falls by lysis. This course sharply distinguishes the disease from typhus, with its brusque onset and termination by crisis. Cases are met with in which the fever is of a remittent type, as in mild cases of enteric fever, or, again, after ten days or thereabouts of high fever, there may be transient remission, to be again followed by another period of high fever of about the same duration.

As in the case in enteric fever the nervous system suffers greatly. In mild cases there may be violent headache, and in severe cases drowsiness, the "typhoid state" or continuous delirium. Restlessness is a notable feature of the disease, especially during the night; patients attempt to rise from the bed, there are involuntary evacuations, and constant watchfulness is necessary. It is characteristic that this serious nervous disturbance appears relatively late in the course of the disease, when the fever has been at its maximum for several days, and that these disturbances continue even when the temperature is falling. In many cases it is only in the afebrile period that the mental state returns to normal.

I do not know whether these symptoms have been noted in kedani fever in Japan, but they are eminently characteristic of the disease as it appears in Deli.

CHANGES IN THE BLOOD

A moderate leucocytosis from 10,000 up to 12,000 per cubic millimeter is the rule, but cases in which the leucocytes number as many as 26,000 per cubic millimeter or as few as from 4,000 to 5,000 per cubic millimeter have been noted.

More significant, however, than the total leucocyte count is the relative proportion of the different varieties. Where there

is no bronchitis or pneumonia, a diminution of polymorpho-nuclear forms and an increase in lymphocytes are almost constantly found. This change is more marked toward the end of the disease when the polymorphonuclear forms may number less than 8 per cent and the lymphocytes as much as 86 per cent of the total. One finds a large number of immature lymphocytes recognizable by their large size and their nucleus. These forms resemble the myeloblasts of Nageli or the "Lymphoiden Mastzellen" of Türck. The remarkable lymphocytosis is, perhaps, due to the general involvement of the lymphatic glands met with in the disease.

The polymorphonuclear leucocytes show the degenerative changes described by Schilling and Torgau. Their number rises immediately when lung complications arise. Eosinophiles may be as few as 0.25 per cent, but they do not disappear altogether as is the case in enteric fever.

Tables II and III show the blood counts in typical cases.

TABLE II.—*Showing blood counts in a typical case.*

Case No. 2071.	Total leucocytes.	Polymorphonuclear.	Lymphocytes.	Eosinophiles.	Mastzellen.	Large mononuclear.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Sixteenth day of disease	6,200	14	76			5
Tenth day of disease	10,000	8	86			6
Seventeenth (temperature has fallen)	5,250	25	68			7
Twenty-second (convalescent)	4,400	34	58	5	1	4

TABLE III.—*Showing blood count in a typical case.*

Case No. 2635.	Total leucocytes.	Polymorphonuclear.	Lymphocytes.	Eosinophiles.	Mastzellen.	Large mononuclear.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fourth day of disease	26,300	32	65			3
Seventh day of disease	14,400	36	59	0.2		4
Eleventh day of disease	12,600	27	61			12
Fourteenth day of disease	11,050	14	85	0.4		1
Fifteenth day of disease	12,380	18	77	0.4		4
Twentieth (temperature has fallen)	10,000	28	64	2.0	1	5

OTHER SYMPTOMS

There are no characteristic symptoms associated with the internal organs. Diarrhœa is uncommon. The lungs and bronchi may be involved, and in fatal cases extensive bronchopneumonia has been found. Albuminuria may be present and in severe

cases may last until the fever has subsided or until the rash disappears.

Finally I would mention the occurrence of rheumatoid pains in the smaller joints. This symptom appears sometimes soon after the fever has subsided, but does not last more than two or three days.

Convalescence follows a normal course, but it is usually some time before the patient is completely well.

In regard to special methods of inquiry, in all cases agglutination tests were applied with the patients serum for *B. typhosus, B. paratyphosus A,* and *B. paratyphosus B;* attempts were, also, made to cultivate organisms from the blood on suitable media. These inquiries, as well as microscopic and bacteriological examination of exudate from the initial lesion and of excised glands, were entirely without result and gave no hint as to the etiological factors concerned in the malady. As it was not possible to infect monkeys by injection of blood from cases of the disease, I could not ascertain whether or not a filterable virus played any part in its causation.

In the post-mortem examination of seven fatal cases, I found only such lesions as may be present after pyrexia.

SUMMARY

1. There exists in Sumatra a disease which resembles enteric fever in its general clinical characters, but is clearly distinct from that disease in causation.

2. There is evidence that this disease is transmitted in a manner similar to that which has been demonstrated for kedani fever in Japan.

3. Though the pseudotyphoid of Deli would appear to be a much less fatal disease than kedani fever of Japan, there are yet many points of resemblance between the two diseases.

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TANAKA, K. *Centralbl. f. Bakt.* (1899), **26**, 432.

Fig. 1.   Larva (*Trombidium* sp.).

Fig. 2.   Larva (*Cheyletus* sp.).

PLATE I.

Fig. 1. Primary lesion in the axillary region.

Fig. 2. Primary lesion in the supraclavicular region. Lymphadenitis.

PLATE II.

Fig. 1. Primary lesion, front of leg, showing inflammatory halo.

Fig. 2. The same twelve days later. Healing has begun.

PLATE III.

ILLUSTRATIONS

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REVIEWS

An International System | of | **Ophthalmic Practice** | edited by | Walter
  L. Pyle, A. M., M. D., Philadelphia | member of the American
  Ophthalmological Society | Ophthalmic Semiology and | Diagnosis |
  by | Charles H. Beard, M. D. | surgeon to the Illinois Charitable
  Eye and Ear Infirmary (eye | department) [etc., 5 lines] | with
  thirteen colored plates and seventy-one | figures in the text | Phila-
  delphia | P. Blakiston's Son & Co. | 1012 Walnut Street | 1913 |
  Cloth, pp. i–xii+1–400.

In his preface the author invites attention to the fact that
there is no other separate volume, in any language, devoted
exclusively to differential ocular semiology. In filling this want,
the author has succeeded most admirably. The descriptions
are full, accurate, and what is rarely encountered in medical
literature, readable, while the illustrations, particularly the
colored ones, render the recognition of lesions of the fundus
a comparatively simple matter. Another point in which this
work is worthy of special commendation is the excellent press
work and the large type which render it really a pleasure to
pick up for an evening's reading. One might wish in this con-
nection, however, that there were an omission of the annoying
method of emphasizing words and phrases by the use of heavy-
face type.

W. H. ALLEN.

A Laboratory Manual and Text-book | of Embryology | by | Charles William
  Prentiss, A. M., Ph. D. | Professor of Microscopic Anatomy in the
  Northwestern University Medical School, Chicago | with 360 illustra-
  tions | many of them in colors | Philadelphia and London | W. B.
  Saunders Company | 1915 | Cloth, pp. 1–400. Price, $3.50.

This book represents the latest work on vertebrata embryol-
ogy and is especially designed for medical students. The scope
of the book includes the study of the chick and the pig, with a
short chapter on human embryology. Special comment should
be made on the excellent printing of the reading matter, as well
as the illustrations, which are all splendidly reproduced.

Of particular interest is the chapter on the dissection of the
pig embryo. The author here is introducing a new method for
the study of the embryonic structures and relations in embryos
over 5 or 6 millimeters in length. This will undoubtedly prove

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to be very instructive to the student, who, after a few dissections, can get a better fixed idea of the important embryonic relations. Since most of the medical schools are now equipped with efficient libraries, a bibliography should always accompany the textbook. The author here has neglected to supply one. A bibliography should be included in all modern textbooks, so that a student may, of his own initiative, look up original articles and thereby become better acquainted with the men who have done fundamental work along the same line.

E. S. RUTH.

The Cancer | Problem | by | William Seaman Bainbridge | A. M., Sc. D., M. D. | [5 lines] | New York | The Macmillan Company | 1914 | Cloth, pp. i–xix+1–534. Illustrated. Price, $4.

The author has composed a very valuable book on an interesting and important subject. He has succeeded in making a readable work, which should prove of value not only to the specialist but more particularly to the general practician. The sections dealing with "Prophylaxis" and "The investigation of cancer cures" are especially worthy of commendation. A large bibliography and a full index, also, add to the qualifications of this book.

J. A. J.

A | Manual of Physiology | with practical exercises | by | G. N. Stewart, M. A., D. Sc., M. D. Edin., D. P. H. Camb. | [7 lines] with coloured plate and 467 other illustrations | seventh edition | New York | William Wood & Company | 1914 | Cloth, pp. i–xxiv+1–1132. Price, $4.

The seventh edition of Stewart's Physiology is brought entirely up-to-date. The arrangement of the book has been improved by breaking the longer chapters up into sections. The chapter on metabolism is greatly enlarged and practically rewritten and is worthy of special mention.

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THE TREATMENT OF THE RETROGRESSIVE SKIN LESIONS OF LEPROSY WITH BASIC FUCHSIN [1]

By OSWALD E. DENNEY

(*Culion Leper Colony, Culion, P. I.*)

THREE PLATES

To one familiar with the clinical aspects of leprosy the curative treatment of the secondary skin lesions is one of the most difficult and discouraging problems.

The frequency with which ulcerations occur, either in the course of the disease or from trauma to poorly innervated tissue, and the almost hopeless task of curing these sluggish lesions tax to the utmost therapeutic resources.

A recent inspection of the 3,500 lepers comprising the Culion Leper Colony showed 42 per cent of them to have ulcers of either the hands or feet. Considering that at some time in his life nearly every leper has ulcers, the magnitude of the work required to care for a large number of lepers can be appreciated.

Among the patients presenting themselves daily for treatment at the surgical clinic of the colony, there is a variety of lesions, many of which have remained unchanged for months or years. Most of the lesions, with proper medication, remain clean; those receiving indifferent attention present an entirely different picture. The slovenly habits of the average leper, the scanty clothing accommodated to the hot climate, the unfortunate anæsthesias, and the lowered body resistance coöperate in producing infection which, untreated, frequently results in gangrene. With the loss of pain sense, and frequently the loss of smell, also, the unfortunate leper fails to recognize the seriousness of his condition until the gangrene has invaded better innervated tissue.

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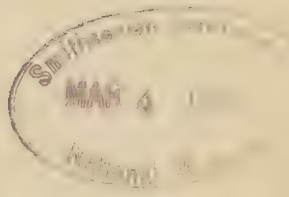
This final condition fortunately is unusual, for while an average of one new case of gangrene is treated daily in the surgical clinic of the colony, the gangrenous process is usually an early and superficial one.

The Filipino leper differs in no way from other lepers in his regard for medicine. A "new medicine" is welcomed for a period, and treatment is regularly received; then interest lags, and it becomes extremely difficult to continue treatment even in the face of encouraging results. Partly for this reason the treatment of skin lesions in individual lepers in Culion has been changed from time to time, but mainly because of lack of improvement with a given prescription.

In the treatment of the chronic ulcerations at Culion the greatest difficulty has been experienced in causing epithelial proliferation. The wounds can be kept clean with a variety of antiseptics, but the epithelial margins of the lesions remain unchanged, giving them sometimes the "punched out" appearance of luetic ulcers.

E. S. May,[2] in an article on the germicidal action of basic fuchsin, presents the following conclusions:

1. From the results of my investigations with basic fuchsin, I conclude that I have a germicidal agent which is more powerful than phenol (carbolic acid) and one which has a greater diffusibility and is less toxic.

2. From my clinical observations I conclude that I have found a germicidal agent which has a marked stimulative action on epithelial and granulation tissue growth.

In a subsequent paper[3] on basic fuchsin in chronic leg ulcers May and Heidingsfeld give a preliminary report on treatment which, while showing satisfactory results, was incomplete because the unauthorized substitution of commercial fuchsin for the more refined fuchsin interrupted their work.

Basic fuchsin as exhibited by May and Heidingsfeld in the treatment of chronic ulcers was either in 1 per cent ointment after the following formula:

Fuchsin (Grübler's Fuchsin für Bakt.)	1 part
Petrolatum	5 parts
Anhydrous wool fat	100 parts

or in gauze bandages saturated with 1 to 1,000 aqueous solution.

Upon theoretical grounds, then, basic fuchsin—a germicide and epithelial and granulation tissue stimulant—should be a desirable preparation in the treatment of leprous lesions.

[2] *Journ. Am. Med. Assoc.* (1912), **58**, 1174–1176.
[3] Ibid. (1913), **60**, 1680–1682.

Accordingly, without regard to the character of the lesions, 132 patients were selected from the members of the colony for experimental treatment.

Since a majority of the ulcers are continuously bathed in serum, it was at the outset considered inadvisable to use ointments, experience having shown this form of medication to be unsatisfactory. Cotton pledgets, soaked in 1 to 500 aqueous solution of basic fuchsin, were packed into the lesions and kept in place by gauze bandages. After a few days the patients complained of a burning sensation in the lesions, and thereafter a solution of 1 to 1,000 was adopted for routine use with little or no discomfort.

The lesions treated may be classified as follows:

    a. Ulcerated tubercles.
    b. Neurotrophic ulcers.
    c. Infected neurotrophic ulcers.
    d. Simple burns.
    e. Infected burns.
    f. Ulcers with sinuses leading to necrotic bone.
    g. Early gangrene (superficial).
    h. Late gangrene (deep).

a. Ulcerated tubercles (Plate I, fig. 1) responded rather slowly to basic fuchsin treatment. Within a few weeks, however, the lesion assumes a pink, healthy appearance, which may continue until the epithelium finally covers the wounds, leaving raised, flat, smooth cicatrices. Of the four cases treated, one eventually repaired, one showed considerable improvement, and two were unimproved after five months of treatment.

b. Neurotrophic ulcers (Plate III, figs. 1, 2, and 3), many of which had resisted treatment for long periods of time, quickly showed improvement. The epithelial margins approximated, leaving smooth, pink cicatrices. The repair, however, not being permanent, subsequent trauma resulted in new ulcers. Thirty-three cases were treated, fifteen of which continued to complete repair, fourteen improved considerably, three did not improve, and one died (from leprous cachexia).

c. Infected neurotrophic ulcers (Plate II, figs. 4 and 5) responded to treatment in a manner similar to simple neurotrophic ulcers, the infection being checked readily and repair continuing. Of the six cases treated, four continued to complete repair, while one died of meningitis and one from septicæmia (each of these two patients was extremely ill when treatment was started).

d-e. Simple and infected burns are very frequent lesions in

the anæsthetic form of leprosy, and of the four cases selected for treatment all quickly repaired.

f. Perforating ulcers (Plate I, figs. 2 and 3), more particularly those of the hands and feet, are usually the openings for sinuses leading to necrotic bone, it being almost impossible to close the ulcers as long as the necrotic bone exists. In a few cases simple curettage of the bone led to speedy repair, but in cases in which all the bones of the foot or the hand were affected, no surgical interference was made, the condition usually being painless and the member being serviceable even in its maimed condition. Of the fifty-three cases treated, twelve were completely repaired, thirty-one improved, nine did not improve, and one died (from leprous cachexia).

g. Superficial gangrene results frequently from extensive burns or from ulcers which are not regularly dressed. In such cases basic fuchsin acted promptly and efficiently. After twenty-four hours no odor could be detected in the wounds, and the progress became checked; after forty-eight hours much of the necrotic tissue could be curetted away; and after seventy-two hours evidence of repair could almost always be seen. Of the 8 cases treated, all repaired promptly.

h. Late gangrene (Plate II, figs. 1, 2, and 3), which sometimes exists several days before the patients call for assistance, responded satisfactorily to fuchsin treatment. Prompt curettage of the superficial necrotic tissue and the packing of the wound with 1 to 500 aqueous solution of basic fuchsin in most cases checked the progress of the disease. The treatment, however, was useless when the patient had already become delirious, or when hyperpyrexia existed. Of the fourteen cases treated, thirteen repaired and one died from septicæmia.

In this series of cases basic fuchsin as antiseptic has proved most satisfactory; as an epithelial and granulation tissue stimulant it has brought about repair in many cases which have resisted a host of medications. Aside from the slight burning sensation from the stronger solutions neither discomfort nor toxic effect was noted.

As basic fuchsin proved to be of great value in the treatment of leprous lesions, it had been adopted as a routine medication even before the completion of the observations on the 132 cases treated experimentally. Several hundred lepers are now receiving this treatment daily, with results approximating those reported in this series of cases.

TABLE I.—*Résumé of cases treated with basic fuchsin at the Culion Leper Colony.*

Lesions.	Re-paired.	Im-proved.	Not im-proved.	Died.	Total.
Ulcerated tubercles	1	1	2	0	4
Neurotrophic ulcers	15	14	3	1	33
Neurotrophic ulcers, infected	4	0	0	2	6
Simple burns	10	0	0	0	10
Infected burns	4	0	0	0	4
Ulcers with sinuses	12	31	9	1	53
Early gangrene	8	0	0	0	8
Late gangrene	13	0	0	1	14
Total	67	46	14	5	132

ILLUSTRATIONS

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Fig. 1. Ulcerated tubercles of arms and legs.

Fig. 2. Perforating ulcer of sole.

PLATE I.

Fig. 3. Same as fig. 2.

Fig. 1.   Gangrene of hand.

Fig. 2.   Same as fig. 1.

Fig. 3.   Same as fig. 1.
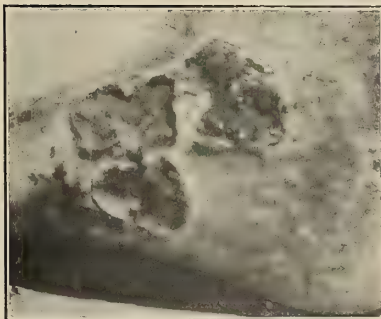
Fig. 4.   Large confluent ulcers of foot.
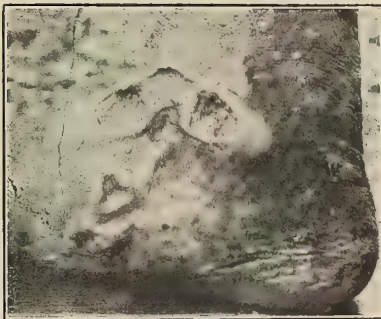
Fig. 5.   Same as fig. 4.
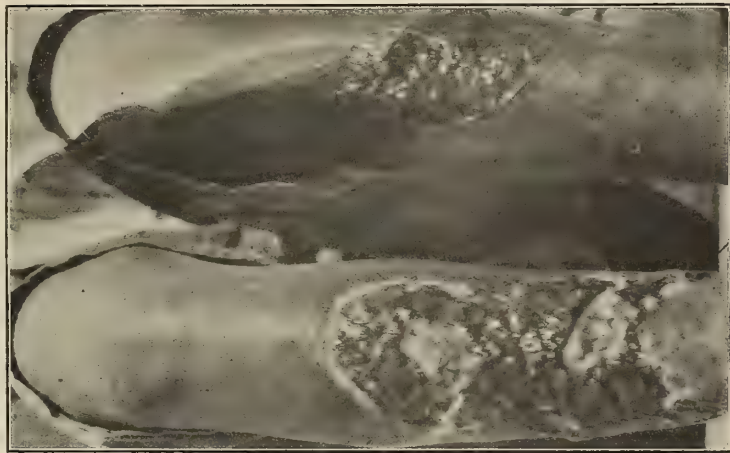
PLATE II.

Fig. 1. Large ulcers of both legs.

Fig. 2. Same as fig. 1.

Fig. 3. Same as fig. 1.

PLATE III.

LEPROSY [1]

By John A. Johnston

(*From the Bacteriological Laboratory, Bureau of Science, Manila, P. I.*)

Leprosy may be defined as an extremely chronic, practically incurable disease, with a long incubation period of from two to ten years, according to various authorities. Prodromal symptoms are stated to exist, but they can hardly be definitely stated to be peculiar to leprosy, as they are usually so indefinite, consisting of malaise, attacks of fever, sweating, and somnolence.

Two well-defined types of the disease exist: namely, the nodular or tubercular and the anæsthetic. A combination of these two may give rise to a third division—the tuberculo or maculoanæsthetic or mixed.

Leprosy existed throughout the world several thousand years before Christ. A disease is mentioned in the Hindu sacred writings and also in the Ebers papyrus which is similar to what we now call leprosy. Ancient writers undoubtedly confused other skin diseases with leprosy, but it has been well established that leprosy was introduced into Europe from Egypt in the first century before Christ by the returning troops of Pompey. The returning crusaders in the fourteenth century spread the disease generally over Europe. It is stated that in France alone there were over 2,000 leprosaria. Very drastic segregation seems to have been established, so that by the end of the fifteenth century the disease had practically disappeared. At the present day in Europe there are a few isolated cases in Norway, Sweden, Turkey, the Balkans, France, Germany, Spain, and the British Isles. Outside Europe leprosy is found in Africa, which is heavily infected, China, Japan, India, the Philippines, the United States, Hawaii, and in many of the islands of the Pacific Ocean.

In the Philippine Islands the records show that the disease was introduced from Japan. The early missionaries from the Philippines had made strenuous endeavors to obtain a foothold in that country. The Emperor finally advised the church

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[1] Read before the Manila Medical Society, July 12, 1915. Received for publication October 2, 1915.

authorities in Manila that he would send a shipload of Japanese for them to experiment upon and endeavor to convert. The good padres were highly delighted, but their pleasure was turned to distress when it was found upon the arrival of the ship in 1633 that the new recruits consisted of 150 lepers. They were permitted to land, but no attempt at segregation was made, and so these people scattered throughout the Islands, spreading infection wherever they located. According to estimates made by the Franciscan fathers, there were at one time 30,000 lepers in the Archipelago. This number was probably greatly exaggerated, as in 1902 the Bureau of Health began making a systematic census of known lepers and reported an estimate of 10,000, probably much less.

During the Spanish régime all leper hospitals were conducted by the church. No very definite policy seems to have been followed. The first leper hospital in Manila was established in 1633 by the Franciscan order as a separate department of their general hospital and was located across the street from the present United States Army Department Hospital. Lepers were here cared for except, for an indefinite period, between 1662 and 1681. At this time Chinese pirates threatened an invasion of Manila, and the patients were moved to Quiapo for safety. In 1784 the church authorities deemed it best to move from Calle Concepcion, and the Government gave them the present San Lazaro estate with the proviso that a portion was to be used for the location of a hospital and the bulk of the estate rented and the money so obtained devoted to maintenance. Portions of the original structure are still standing and are, I believe, in use as the leper department of the present San Lazaro Hospital.

In 1859 Pedro Felix Huertas took charge of the hospital; he seems to have been a very excellent executive and to have made many improvements. The stone walls, which exist to-day, were built by him at a cost of 30,000 pesos.[2]

At the time of the American occupation there were three hospitals devoted to the care of lepers: namely, the San Lazaro in Manila, one in Cebu, and another in Ambos Camarines, the total capacity of the three being estimated at 400.

In the report of the former Board of Health (now Bureau of Health) for 1902 mention is made of the necessity for establishing a leper colony. A committee appointed by the military

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[2] One peso Philippine currency equals 100 centavos, equals 50 cents United States currency.

authorities had reported favorably upon Cagayan Sulu Island, but had overlooked the question of a proper water supply. The Philippine Commission appointed a committee to make a new survey, and Culion Island was decided upon as a good site. It was not until 1906 that lepers were sent to the colony. The first colonists numbered 500, and at the present time some 3,000 persons are living there.

<center>ETIOLOGY</center>

Leprosy is caused by an acid-fast bacillus—the so-called bacillus of Hansen, or *Bacillus lepræ*—which resembles the tubercle bacillus both as to morphology and staining peculiarities. It is a slender rod, 2.5 to 3.5 microns in length by 0.3 micron in thickness. It is usually straight, but sometimes slightly curved, and occasionally clubbed forms are seen. It is nonmotile, and is not definitely known to produce spores. I have often seen, however, in smears from leprous nodules, small rounded bodies 0.5 to 2 microns in diameter, which are distinctly acid-fast, and have noted that where these exist the typical leprosy bacilli will also be found after careful search. Leprosy bacilli are found in the tissue cells in the lymph spaces, and while isolated organisms are frequently noted, a characteristic grouping of from two to three to many individuals in a bunch or bundle may occur. This has not been inaptly likened to a package of cigars.

A word as to the differentiation between the bacillus of tuberculosis and that of leprosy. The leprosy bacilli are usually present in large numbers, often packed in groups, or bundles, in the juice squeezed from a leproma or in scrapings from the nasal mucosa, provided an ulcer of the septum exists. In tubercular skin lesions it is the exception to find a single bacillus in juice obtained after such a procedure. The leprosy bacilli are said to be less acid-resisting than the tubercle bacillus. This fact is in a measure true, but I have often found typical leprosy bacilli that were as acid-fast as the tubercle bacillus.

It has been stated by several authors that the leprosy bacillus stains more solidly and when granules are present they are coarser and more widely separated than the fine granulations of the tubercle bacillus. The bacilli are chiefly spread by the lymphatics, but they may be found in the blood stream in the nodular type of the disease. The following procedure should be used: Puncture a vein and permit the blood to flow into 10 per cent acetic acid solution in the proportion of 1 to 10, allow it to act from one to two hours at 37°C., and then centrifuge. Very beautiful specimens may be secured in this way.

It is a well-known fact that in cases of the nodular type recrudescences apparently occur at varying intervals, to be followed by feverish attacks with accompanying swelling and congestion of the nodules and a new outcropping of lesions. This condition is well recognized by the lepers at Culion, and in the Tagalog language the condition is termed *alabajar*. I have not had the opportunity to make repeated examinations in these cases, but the symptoms suggest a true bacteræmia.

It is generally believed that each case of leprosy takes origin from contact with another case for a varying period of time. We have no definite knowledge at the present time as to the exact method of transmission, but one well-established fact seems to be that intimate contact is necessary. In adults such contact can easily be accounted for by the sexual relation, and in children by familiar relations which are more or less intimate. Even with such close contact, infection is rare, as is shown by the following figures taken from a report on the health conditions at Hawaii. Of 225 healthy natives living in the same house as lepers only 4.5 per cent acquired leprosy. Among the married only 9 out of 181 contracted the disease from their leprous wives, or husbands, as the case might be.

Many writers have endeavored to involve the insect world as bearing an epidemiologic relation to the disease. Thus flies, bedbugs, cockroaches, head lice, mites, and ticks have all been hailed as carriers and conveyers of leprosy bacilli, but there is still no proof of actual guilt. When we stop to consider the ubiquity of acid-fast bacilli in nature, and the habits of feeding of these insects, one wonders if almost all of the reported positive findings of leprosy organisms in different insects are not errors. My own experience in this line consists in one series of observations. Three hundred fifteen bedbugs were collected at Culion from the beds in hospital, from houses, and from clothing. Fifteen of these bugs were examined individually by dissecting out the mouth parts and the intestinal tract, crushing between slides, and making a smear. Acid-fast bacilli were found in the intestine in one instance. The remaining bugs (300) were ground up in lots of 50 in sterile salt solution and centrifuged, and smears were made. An average of 100 smears was examined from each lot. Acid-fast bacilli were found four times, making a total of five times for the entire number of bugs.

Much stress has been laid by various writers on the importance of examining the nasal mucus, but unless there is a leprous ulcer of the septum, this is of little significance. The mere presence

of acid-fast bacilli in nasal mucus should not be regarded as diagnostic of leprosy in the absence of clinical signs.

The diagnosis of leprosy may be considered under clinical and microscopical heads.

CLINICAL

The patient should be in a good light, and at first the examiner should stand some distance away, as by so doing he will often be able to distinguish certain areas which in the dark-skinned races show up only at a distance, such as dusky reddish patches, which on a near view one cannot differentiate at all from the surrounding skin. Fixing these patches in mind, they should next be palpated; usually a certain degree of inelasticity will be felt. Such a patch is less than an induration and is apparently not due to any subcutaneous œdema or effusion, but rather to an atrophy of muscular tissue. In old cases the skin will remain after being pinched up. In early cases these areas are excèptionally greasy, but later on dry, and frequently they do not sweat. Tests for anæsthesia should always be made in these spots. The center of the area is usually more anæsthetic than the borders. One should also look for loss of eyebrows, nodules in the ears—or, in old people, for elongation of the pinna—and nodules in the nasal alæ. In leprous skin lesions there does not seem to be a true pigmentation, as what is often called pigmentation is really a loss of natural color as compared with the surrounding area. The most confusing diseases which are frequently mistaken for leprosy are syphilis, lupus, yaws, mycosis fungoides and psoriasis, elephantiasis, and madura foot. Some authors have cited syringomyelia as being especially difficult of differentiation. In a personal experience covering several thousand cases I have never seen a case of this disease.

MICROSCOPICAL

Success in detecting the leprosy bacillus depends largely on the method of taking the specimen. Selecting a nodule or the edge of an indurated area previously circumscribed with a sharp scalpel, one or more slight incisions should be made just through the true skin. If the incision is properly made, only a small amount of blood will appear. This is wiped off, and the serum which exudes is taken up by the blade of the scalpel; it is well to scrape the edges of the incision. The whole should be smeared as evenly and thinly as possible on a slide. Dry in air and fix by direct heating over the flame or by dropping one or two drops of alcohol on the slide and setting fire to it.

Stain with carbol fuchsin and heat until boiling. Allow to cool, wash in distilled water, decolorize either with Gabbet's methylene blue and sulphuric acid or with 20 per cent aqueous nitric or sulphuric acid, and counterstain with Lœffler's blue. Personally I do not like a counterstain, but this is a matter of little importance.

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TROPICAL OBSTETRICAL PROBLEMS [1]

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There is not the slightest doubt that since the American occupation of these Islands in 1899 much progress has been made in this country in the different branches of medicine.

If we confine our consideration to obstetrics, the advance in this branch of medical science is still more remarkable. In the past, obstetrical teaching in this country consisted merely in didactic lectures, sometimes demonstrations on the manikin, and of two or three cases of labor during the school year, and it frequently happened that the students were graduated without having seen a single obstetrical case. Owing to these circumstances many physicians, when called upon to attend an obstetrical case, were very much afraid to meet any complication that might arise during labor, and when a physician whose inclinations were for obstetrics wanted to develop his knowledge in order to be a specialist in this line, he had to go abroad, as there were no facilities here for special work.

In July, 1907, the College of Medicine and Surgery was established with its obstetrical department in the city of Manila. At first the obstetrical clinics were held provisionally in a small room in Saint Paul's Hospital with only four beds, and two municipal physicians were appointed to attend outside obstetrical cases. At that time we had very few patients, the majority of whom were outside cases, because, in general, the Filipino women were opposed to entering the hospital, their habits and customs being to deliver in their own houses, and also, because they were imbued with many superstitions and were accustomed to their own practices. I think it will not be out of place in this connection to present a list of superstitions relating to obstetrics, which I have carefully collected from the ignorant classes of our people.

BEFORE THE BIRTH OF THE CHILD

DURING PREGNANCY

During pregnancy it is believed that the asuang has a great influence upon the pregnant woman. The asuang is supposed

to be a very active evil spirit, troublesome alike to men and women, especially to women who are pregnant or in labor, and also, to new-born children. The asuang resembles the vampire of Europe, and is believed to suck the blood of human beings while they are sleeping. This belief causes pregnant women to be careful, for fear they become its prey.

The asuang is believed to be more troublesome at night than in the daytime, and for this reason pregnant women endeavor to remain in the house at night. Should occasion require them to leave the protection of the house, it is customary for them to let down the hair, as in so doing the asuang is frightened and dares not approach the woman for the purpose of transforming the prospective child into another asuang. It is, also, customary to keep a light or fire burning at night beneath the house, as these evil spirits are believed to be frightened away by this means.

The asuang is supposed to be a very clever spirit, but it is unable to see any person who is covered by a cloth, and pregnant women are always advised to sleep under a black blanket during the last months of pregnancy.

In the presence of a pregnant woman it is considered unlucky to cross a halter, or rope, attached to a horse or any other animal, as a difficult labor will be caused.

Pregnant women are not allowed to eat *tutong,* or rice crust, because by so doing the expulsion of the placenta will be delayed.

When a pregnant woman voids urine on the ground, it is necessary for her to pour water over the place where she urinated, otherwise the asuang might smell the urine, and the woman will have hæmorrhage at the time of labor.

Pregnant women should not carry coins under the folded waistband of their skirts, because if they do so the child will have tumors.

When engaged in cooking, they must be careful to place the wood in the stove large end first, otherwise the baby will be born in the opposite direction—that is, a breech presentation will result.

During the last three months of pregnancy what is termed *pagbubungkal* is performed, which consists in turning the baby in order to place it along the median line of the mother. This is a very dangerous practice.

DURING LABOR

On arrival the midwife rubs the body of the parturient with her hands.

The yolk of eggs is then administered in order to give strength. Sometimes uncooked cocoa, pure chocolate, or human milk is given, in order, it is said, to facilitate labor. The midwife never allows any one to stand in the doorway; otherwise the child will stop at the pelvic outlet.

During labor the asuang may, also, do harm to the parturient woman, and in order to keep it away, a light is kept burning under the house all the time. Sometimes this precaution is considered insufficient, and in this case, the doors and windows are smeared with a mixture of garlic and salt, in order to prevent the entrance of the asuang.

To facilitate the birth of the child, the midwives and *mediquillos* resort to the following procedure:

The salag.—This method consists in locating the fœtus by palpation, and by pressing and pushing, causing it to slide down against the abdominal wall, thus making labor easier. These manipulations are usually performed by a strong man or woman, preferably a man, who makes compression and expression of the uterus with his hands or with a piece of wood in such a manner that the abdomen as well as fœtus is often bruised or wounded, and there is a too rapid expulsion of the fœtus and resulting lacerations. Rupture of the uterus itself has occurred under such treatment.

The use of a decoction made from roots of cogon and other plants is believed to facilitate labor.

To stop strong labor pains, the leaves of *bagabaga* are burned near the woman in labor, or twelve buyo leaves are bruised in milk, and the mixture is rubbed over the lower part of the abdomen and the thighs. At the same time the woman is made to take one raw egg and to apply another egg over her abdomen as a poultice.

WHEN THE CHILD IS BORN

After the birth of the child the cord is not cut until the placenta is expelled. The child is, therefore, exposed for several hours sometimes, while waiting for the placenta to be delivered.

When the expulsion of the placenta is delayed, the midwives pull on the cord, while at the same time they push the uterus downward. This method is dangerous, because the cord might be torn off the placenta, or else the placenta might be forcibly detached from the uterine wall, leaving behind some cotyledons, or inversion of the uterus may be produced.

When the placenta is delivered, the cord is cut and it is buried in the ground. It is believed to be a bad practice to throw

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it away. A hole is made for it, and care is taken to make it just large enough to fit the placenta, because, if the hole is too big, the baby will become a glutton, and if it is too small, the baby will have a poor appetite and small intestines. Some think it better to throw the placenta in the river, so that the baby will become healthy and strong. Others believe that if the placenta is buried wrapped in paper with a pen or a book the baby will become an active and wise man.

The placenta is, also, used as a medicine. It is cut in pieces and then boiled and given to a primipara to eat, for the purpose of preventing many kinds of diseases during the puerperium.

The child is separated from the placenta by cutting the cord with a knife made of a piece of bamboo, preferably *boho,* but sometimes with a dirty bolo or a pair of scissors. The cord is then coiled, dressed with ashes, and finally wrapped in a piece of linen or paper, which is usually dirty.

If the cord is cut in pieces and hung on the eaves of the house, it is believed to prevent the diseases of childhood.

In some portions of Luzon, as in Nueva Ecija, for example, before dressing a male child a barbarous maneuver is performed, which consists in fracturing the penis by folding it over at its middle. The reason for this is to prevent the child becoming effeminate. There is a commoner but less dangerous practice. It is what is called *minamainitan,* which consists in heating pieces of flannel or other cloth and then applying them hot over the abdomen, umbilicus, and sexual organs of the child. If the child is a male, they rub the cloths upward over the sexual organs to prevent hernia. Leaves of *romero* may, also, be used for this purpose.

After cleaning and dressing the baby, the midwife administers a purgative, or a bitter juice, as the juice from *alpalea,* which is sometimes mixed with a few drops of the mother's milk. The juice is produced from the leaves, the number of which must always be uneven in order to be effective.

The baby is wrapped in such a way that both hands and feet may not move, and it is not allowed to nurse for two days.

After the birth of the child the mother is transferred to a clean mat and then the operation called *pagkaban* is performed by the midwife and her assistant, or salag.

The operation of pagkaban is performed as follows: The midwife, squatting on one side of the parturient, holds the hands of her assistant (salag), who is in the same position on the other side. Then they compress the woman's pelvis with their feet, pulling each other's hands as they do so in order to return

the bones to their normal position.   Finally, massage and irrigation are given and repeated every afternoon.

<div align="center">AFTER LABOR</div>

As soon as the placenta is delivered, a binder is tied very tightly around the waist of the patient.   The object is to prevent the entrance of air into the abdomen, in case the woman takes a deep breath, and also, to prevent the blood from going up into the head.

If there is postpartum hæmorrhage, the hair of the patient is tied very tightly on the top of the head in order to stop the bleeding.   This remedy is, also, used as a prophylactic treatment for hæmorrhage.

Another remedy for hæmorrhage is heat, produced by burning bamboo in the form of *sulo,* or a torch, under the house.   There is still another remedy by filling the vagina with *kayas,* or cuttings of bamboo.

The day after labor the woman begins to suffer from a series of maneuvers performed on her by the *hilot,* or midwife.

There is what they call *sara,* which consists in compressing the bones of the pelvis, a process which I have already described.   When the woman complains of pain, weakness, paralysis of the legs, or prolapse of the uterus after labor, it is because the sara has not been made or because it has not been well done.

The massage of the abdomen, which the hilot performs with caution, continues every day for eight days.   After each massage the tight binder is put back in place.

In some places, as in the Ilocano and the Tagalog provinces, the people perform the *saklap,* or *salap,* which consists in giving the woman a steam bath to produce a profuse perspiration. Many women become anæmic from this treatment, and if the condition of the woman after the treatment is bad, the hilot says that she has not been properly "cooked."

Bathing.—After ten days a bath is given.   On the preceding afternoon leaves of certain plants, such as *talbak, tanlad, lagundi, suha, galamayamo,* and *sambong,* are collected and boiled together in a kettle.   The next morning a bath is given with this infusion, while the leaves are all taken from the kettle and placed upon the mat where the woman sits in a squatting position.

The leaves are again boiled and then covered with banana leaves to prevent evaporation.   This is used for the operation called *pagkukulob* as follows: After lunch and a little rest the

woman is wrapped from the neck down with a buri mat, and then she is asked to stand with feet wide apart over a kettle of hot water covered with banana leaves. While the woman is in this position, a hole is made little by little in the banana leaf cover in order to allow the steam from the kettle to heat the external organs and the whole body.

The bath after labor is taken in various ways. In some places it is taken only when there is no more lochial discharge. In others, as in the Ilocano provinces, immediately after labor the woman takes a bath and she does so every day for six days. After each bath she goes near a *kalan*, or a stove, and she remains there all day to warm up her hips. In San Fernando, Union, the woman just delivered takes as many baths as the number of days that have passed since the time of labor. For example, one bath on the first day, two baths on the second day, etc., until the ninth day when she takes nine baths.

Every afternoon the abdomen is massaged with a heart-shaped stone or a piece of iron about 10 centimeters long, prepared for this purpose, which is heated and then wrapped with leaves of *pandakaki*.

Any puerperal disturbance, such as puerperal insanity, for example, is attributed to the *mangkukulam*, or witch.

DURING LACTATION

The woman must not nurse her baby if she has been cooking or ironing, because her milk has been altered by the heat (*panis*). If she has not nursed her child for several hours, her milk becomes bad, also, and so she must remove the supposed bad milk first before giving her breast to the child.

Anything that is sour is bad for a nursing woman, because it coagulates the milk and the baby will have colic.

She must, also, see that no lizard drinks her milk, otherwise the secretion of milk will be stopped.

Hyperlactation is prevented by the use of a key as an amulet or a few papaya flowers suspended from the neck.

OBSTETRICAL PROBLEMS

As I have already said, we had, at the beginning of the obstetrical department, only a few cases, not more than 10 a month, consisting of the worst cases in the city—those suffering from the misguided attentions of ignorant midwives and friends. The large majority of the patients refused to be confined in the hospital; consequently the work had to be done in the homes of the poor, which were usually in a most unsanitary condition and

where it was sometimes even impossible for us to obtain clean water to use for the patient and her baby. The patients were, in the majority of cases, so poor that they could not afford to furnish even basin, soap, or towel.

In spite of all these difficulties students were taught to conduct labor, make application of the forceps, and perform podalic version and other operations.

The death rate was high, due to the ignorance of the Filipino women in matters of hygiene and to the fact that they were opposed to calling in a physician, except when they were already in a very serious condition, and when it was impossible or almost impossible to do anything for them. With the object of enlightening the people, several movements were begun in the department to improve the service and to educate the people, such as the organization of the out-patient service with resident physicians and internes at the hospital, the distribution of pamphlets and letters among the poor Filipino women in Manila, and lectures and conferences in the different health districts of the city.

These lectures and conferences dealt chiefly with those subjects pertaining to the pregnant woman, care of children, and midwives, and we always tried, in so far as possible, to gather at our meetings the pregnant women and midwives of the district. Our work was satisfactory, as it resulted in the gradual increase of our cases and decreases of our death rate, especially in the out-patient department, as shown in Table I.

TABLE I.—*Obstetrical cases from 1907 to 1914, showing increase of hospital and decrease of outside service.*

Year.	Deliveries and puerperium.		Abortions.	
	Hospital.	Outside.	Hospital.	Outside.
1907 to 1910	110	454	10	24
1911	241	282	21	6
1912	336	515	46	8
1913	513	373	3	1
1914	567	316	45	7
Total	1,767	1,940	125	46

It is only just, however, to state that the success in educating the people in the advantages of hospital care was not entirely, although it was mainly, due to the activities of our department, because we have, also, to consider the influence of other insti-

tutions, as an example the Mary J. Johnston Hospital, where Filipino women learned that hospitalization was a good thing for them, be they in normal or abnormal labor. In January, 1911, or a little over four years ago, the obstetrical department was transferred from the small room in Saint Paul's Hospital to floor 11 of the Philippine General Hospital, where we had better facilities for obstetrical work of all kinds. This transfer of the maternity department to a modern and well-equipped institution contributed very considerably toward attracting women to be subjected to hospital treatment, not only because they saw that the new maternity ward was excellent in the matters of hygiene and afforded an ample space for a large number of patients, but also, because they saw that we had all the facilities that were necessary, especially in cases needing intervention. As a result of all these influences that contributed to the education of the Filipino women, we have been able to increase the number of confinement cases with medical assistance in the city of Manila from 5 per cent to 70 per cent.

The department of obstetrics was organized in July, 1907, in Saint Paul's Hospital, and from that time to December, 1914— that is, a period of seven years and a half—we have had 3,707 cases of deliveries and puerperium and 171 abortions, with the complications, deaths, and operations shown in Table II.

TABLE II.—*Classification of 3,707 delivery and puerperal cases from July, 1907, to December, 1914.*

	Cases.			
	Hospital.	Outside.	Total.	Per cent.
Complications:				
Placenta prævia	125	15	140	4
Eclampsia	125	8	39	1
Puerperal infection	115	4	119	3
Operations:				
Forceps	158	69	227	6
Podalic version	121	17	138	3
Embryotomy	15		15	
Cæsarean section	16		a16	
Laparotomies for abdominal pregnancy			2	
Maternal deaths:				
Placenta prævia			36	25
Eclampsia			18	45
Puerperal infection			19	15
Other causes			84	
Total			157	4

a Two post mortem.

We have had 4 per cent of placenta prævia, as against 1 per cent of eclampsia, which shows that placenta prævia is more frequent in this country than eclampsia, a fact which is just the opposite to what I have observed in several clinics of France and America, where eclampsia seems to be more frequent than placenta prævia. Placenta prævia occurs in the majority of cases in multiparæ, and the reason why it is common in this country is, to my mind, due to the defective management of previous labors which almost always give rise to many kinds of uterine diseases and displacements which favor defective implantation of the placenta during the development of the fœtus.

In regard to puerperal infection we have 3 per cent, which is not high, due to the fact that we had practically no cases with puerperal infection except those brought from outside, who came after they had already been infected, either during a prolonged labor attended by midwives and friends, or during the puerperium. In the hospital parturient women who come before or at the onset of labor do not develop puerperal infection, as a rule, and those who become infected develop only a mild type of infection.

The great majority of forceps applications has been for inertia of the uterus and in a few cases of contracted pelvis. Podalic version is resorted to in all cases of transverse presentation when the fœtus is alive and, also, when the fœtal head is high and not engaged in the pelvic inlet. We found from experience, however, that podalic version is not always a safe procedure in cases of transverse presentation, as when version is made several hours after the rupture of the amniotic sac, and the fœtus is already dead, there is almost always danger of rupturing the lower segment of the uterus, leading to postpartum hæmorrhage, peritonitis, or infection. For this reason we have made it a rule in our practice in the hospital to resort to embryotomy in all cases of neglected transverse presentation—that is, when the uterine cavity is already drained of its amniotic fluid and the fœtus is dead. Also, in prolonged labors due to contracted pelvis, or large fœtal head, instead of applying forceps and other measures, we perform craniotomy as soon as we determine that the fœtus is dead. We have performed Cæsarean section in all cases, except one, on women with placenta prævia, the exception being a case of intrapartum eclampsia in a primigravida. In this case Cæsarean section was the best way to extract the fœtus, as the cervix was not dilated and rapid delivery was indicated. The

performance of any other operation, such as the dilatation of the cervix by metal dilators and balloons, would require at least one or two hours and then there would be the additional risk of rupturing the artificially dilated lower segment of the uterus if forceps application or podalic version was made in an attempt to deliver the child. We found from the result of our observations that Cæsarean section is the best and safest procedure to follow in all cases of placenta prævia where the cervical canal is not widely dilated, because we can prevent in this way the loss of much blood which would surely result if the cervix were to be forcibly dilated for the extraction of the fœtus through the parturient canal. In Cæsarean section we have always obtained good results in cases of placenta prævia and eclampsia, except in cases where the patients come in bad condition; these usually die, no matter what kind of intervention is made.

Some authorities claim that labor subsequent to the performance of Cæsarean section is dangerous, citing several cases where the uterus has ruptured in the scar of the uterine incision. Three of our patients on whom we performed Cæsarean section for placenta prævia have returned to us for delivery, and in these three cases labor was perfectly normal in every way, thus proving the conclusion that Cæsarean section does not predispose to rupture of the uterus during labor as long as the suturing of the uterine wound is properly made to effect good and complete healing.

Among our cases there were two abdominal pregnancies, one of which was interstitial ovarian pregnancy, while the other was interstitial uterine pregnancy. To extract the fœtuses, which were alive, laparotomy was performed in both cases.

A few cases of contracted pelvis necessitated the application of forceps, but none of them required the performance of pubiotomy, Cæsarean section, or the like. The reason is that a contracted pelvis seems to be just as rare here as in other countries, although it is true that, compared with the pelvis of white women, Filipino women have small pelves. The size of the Filipino woman's pelvis has attracted our attention since the department was organized, and although we made it a routine practice to take the external measurements of every parturient both in the hospital and outside, we did not begin to make a more systematic determination of the average external and internal measurements of the female pelvis in this country until last year. Our work along this line is still going on, and therefore I am not in a position to offer any final conclusion; but in order to give an idea of the difference between our measurements and those given

in textbooks, I present some of our findings, which are based upon the measurements of 300 pelves.

TABLE III.—*Comparative measurements of the pelvis in Filipinas and Americans.*

	American.	Filipino.	Difference.
	Cm.	*Cm.*	*Cm.*
Diameter between the iliac spines	25.47	23.90	1.47
Diameter between the iliac crests	27.99	24.90	3.08
Diameter between the trochanters	30.90	28.10	2.79
Baudelocque	19.71	17.63	2.08
Diagonal conjugate	12.26	12.00	0.26
Anteroposterior diameter of outlet	12.50	10.05	2.44
Transverse diameter of outlet	11.00	11.00	----------

As can be seen, the pelvis of the Filipina is smaller than that of the American or the European in all the diameters except in the tranverse diameter of the outlet where they are in the same proportion. I cannot go into details, however, in the consideration of this subject, as our investigation is not as yet complete, but one of the principal reasons why the Filipinas have small pelves is because the Filipinas are small in stature, and their pelves are in proportion to their size. In measuring the heads of 260 new-born babies, we found that the cephalic diameters of Filipino babies are smaller than those of the American.

TABLE IV.—*Comparative measurements of heads of new-born babies of Filipinas and Americans.*

Diameter.	American.	Filipino.	Difference.
	Cm.	*Cm.*	*Cm.*
Occipitomental	13.33	12.11	1.22
Occipitofrontal	11.70	10.96	0.74
Sub-occipitobregmatic	9.70	9.28	0.42
Biparietal	9.25	8.63	0.62
Bitemporal	8.00	6.82	1.17

This diminution in the diameters of the fœtal heads in this country can, of course, be accounted for by the small size of the pelvis of the Filipino mothers—that is, it is due to the law of pelvic accommodation. It is, therefore, important to bear this in mind, else we might be lead to resort to some drastic measures when we happen to have on hand a difficult case of labor in a Filipino patient and when we find that her pelvic measurements are less than those given in the textbooks.

The general death rate of 4 per cent is based upon the result of our work from the beginning of the institution, and therefore it is not the present death rate of our cases in the Philippine General Hospital, which is, of course, considerably less.

Obstetrical teaching in this country used to be deficient in the extreme, as I have already stated, due to the fact that the instruction was entirely didactic and the students could, therefore, manage normal labor and perform obstetrical operations in theory only; but since the opening of the department of obstetrics of the former Philippine Medical School, which has now become the College of Medicine and Surgery of the University of the Philippines, modern methods of instruction are in use, and the students are now given not only lectures, but also actual, practical demonstrations on the pregnant, parturient women. Besides demonstrations, the students are permitted to assist in all normal and operative cases of labor, and they are allowed to deliver normal cases in the presence of one of the residents of the department.

The course in medicine in our university lasts five years. Obstetrics is taught beginning in the fourth year and continuing through the fifth or senior year. During these two years the students are required to be on duty, in rotation, for twenty-four hours in the Philippine General Hospital, ready to be called at any time to attend, together with the obstetrician on duty, all cases of labor both in the maternity ward and in the out-patient service of the department. In 1914 we had 883 delivery and puerperal cases and 52 abortions. As there were only 21 fourth- and fifth-year students, and all our patients have practically been attended by them, it is safe to assume that each student has seen at least 42 cases of labor, some of which he delivered under the supervision of one of the members of the staff. The training of our students, therefore, compares favorably with that of the medical students in other up-to-date universities, if it does not give them advantage over the latter, as in most of the other universities the students do not usually have the opportunity to see so large a number of normal and abnormal cases, the variety of which as well as the number of operations performed I have already enumerated. As a result of this new procedure in obstetrical teaching our students are already equipped, before graduation, with a sufficient practical knowledge of those matters which they will likely meet in private practice.

From all that I have explained, we can conclude that the solution of the obstetrical problems in the city of Manila is at the point of complete realization, as the women have already learned the advantages of medical assistance. It is now a common thing

to see them go to the hospitals for confinement or to call physicians, nurses, or qualified midwives to attend them in their homes. However, it is necessary to remember that the strongest attraction for them is the free medical assistance given by the Government in the Philippine General Hospital and in the out-patient service of the Department of Obstetrics of the College of Medicine and Surgery, and in order that a larger number of women may be attended in these two maternity services, it is necessary to increase our facilities, enlarging the maternity ward of the Hospital and increasing the appropriation for maternity work, especially in the out-patient department.   In this way we shall be ready to meet the real needs of the Filipino mothers, having more physicians and nurses to go around the entire city and to handle our cases in the hospital.

Obstetrics in the provinces, however, is an entirely different matter, as there are no influences, such as exist in Manila, to abolish superstitious ideas concerning midwifery.   It is evident that to accomplish such an object there should be provincial maternity institutions with staffs of physicians, nurses, and qualified midwives to show them the modern way of living and of taking care of themselves and their children.   This side of the problem has already been taken up by the Legislature, and as a beginning a law has been passed creating the School of Midwifery in connection with the School of Nursing of the Philippine General Hospital, where young women from the provinces are given a special course in obstetrics to enable them to practice scientific midwifery in the provinces.   When the time comes when the graduates of the School of Midwifery begin to spread throughout the Philippines, and when the provinces are divided into sanitary districts having physicians, nurses, and qualified midwives to look after the health of the people, we shall have accomplished the aspiration of the country, which is to wipe epidemics away from these Islands, to save the lives of a great many parturient women, to solve the very important problem of our high infant mortality, and to make the Filipinos a healthier and stronger people.

A STUDY OF THE PATHOLOGY OF THE GALL BLADDER AND BILIARY PASSAGES IN CHOLERA [1]

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In most of the literature dealing with cholera little or no mention is made of the pathology of the biliary passages and the gall bladder in this disease. Recently several papers have been published on this subject, calling special attention to its importance in relation to chronic and intermittent cholera carriers. This study was undertaken to determine the pathological condition of the gall bladder and the bile ducts of the cholera autopsies in the cholera outbreak in Manila in 1913–14.

Kulescha(1) first emphasized the importance of this subject in relation to the carrier question. In his review of the literature he notes that Pirogoff(2) in 1848 observed two cases of diphtheritic cholecystitis in cholera autopsies, one of which showed perforation of the fundus of the gall bladder and general peritonitis. Netschaeff(3) records a similar case in 1892, also one with acute cholecystitis without perforation, and two that showed catarrhal inflammation in sections of the gall bladder.

There were a number of early observations on the presence of the cholera vibrio in the bile. Nicati and Rietsch,(4) in 1884, examined the bile in three cases of cholera and found the vibrio in two, and later in two of five cases. Similar observations were made by Doyen,(5) Kelsch and Vaillard,(6) Tizzoni and Cattani,(7) Raptschevsky,(8) Rekovsky,(9) and Defressine and Cazeneuve.(10) Sawtschenko,(11) in 1892, found cholecystitis twice among 30 cholera autopsies. In a series of 28 cases recorded by Girode,(12) vibrios were found in 14. One case showed marked symptoms of cholangitis and cholecystitis with vibrios present on bacteriological examination. Brulloff(13) found vibrios in 76 per cent of his cases.

Kulescha,(1) in the cholera epidemic at St. Petersburg in 1908–1909, performed 430 autopsies. He found cholecystitis in 42 cases, or nearly 10 per cent; 21 of these were in the first week of the disease, and 19 were in the second week.

In these cases the gall bladder was brown or yellowish gray, distended, and greatly congested. The mucous membrane was covered with a thick, turbid mucus often mixed with pus. When this was removed, the mucous membrane was seen to be swollen and bright red. In one case he found a great number of small, scattered areas covered by diphtherialike membranes. In eight cases the gall bladder contained a thick, colorless fluid.

Microscopically his cases showed the mucous membrane of the gall bladder denuded of its epithelium and infiltrated with round cells. The submucosa shows a marked round-cell infiltration, dilatation and congestion of the blood vessels, and some blood extravasations. In more severe forms there is a necrosis of the mucosa extending to the submucosa. Kulescha characterizes this as a catarrhal hæmorrhagic inflammation, the same as is seen in the intestines.

Bacteriological examination of the bile showed the vibrio in 40 cases.

In regard to the bile ducts Kulescha found only four cases in the above series in which cholangitis could be recognized grossly. Microscopically these cases showed the bile ducts denuded of their epithelial layer and the wall infiltrated with round cells. The lumen was filled with granular débris, composed of leucocytes, bile pigment, and cells of cylindrical epithelium. Some cases showed necrosis extending to Glisson's capsule and even to the liver cells. This inflammation was more severe in the larger ducts.

Of the four cases, Kulescha(1) describes three as purulent biliary hepatitis and one as hepatic biliary cirrhosis. Bacteriologically cholera vibrios were found in all four, but in only two in pure culture. By appropriate staining methods vibrios were demonstrated in sections from one of these cases. The vibrios found in the tissues were not identical with the ordinary vibrio, but resembled involution forms of the cholera vibrio when grown on agar or potato—that is, thick and swollen.

In this connection Kulescha records an interesting case illustrating the importance of this infection in regard to chronic and intermittent carriers. A woman, aged 36, one year before her

death, was admitted to the hospital with cholera with the usual symptoms as well as marked jaundice. Physical examination showed an enlarged liver. Vibrios were found in her stools at this time and for fifty-seven days thereafter. Seven months later she was admitted to another hospital with enlarged and painful liver. At autopsy, four months later, cholera vibrios were recovered from the bile ducts, but not from the intestines. Kulescha quotes this case to explain the intermittent cholera carriers. The liver showed a marked biliary stasis due to the cholangitis. Therefore the bile containing the vibrios only reached the intestines at intervals.

Grieg(14) records the largest series of bacteriological examinations of the bile for cholera vibrios. He examined 271 cases and found the vibrio in 80. In 12 (4.4 per cent) of these there were distinct pathological changes. One of these cases was recorded in detail in 1912(15) and another in 1913.(14) In the latter case the gall bladder was shrunken and contained a small quantity of dirty, brown bile. The mucous membrane was congested. Histologically the sections stained for the vibrios showed their presence not only in the mucosa, but also deep in the submucosa.

In another article(16) Grieg tabulated the results of 235 cholera autopsies at the Medical College, Calcutta. Ten cases (4 per cent) showed to the naked eye some signs of inflammation of the gall bladder. He notes that the macroscopic changes observed in the gall bladder were the following: Slight thickening of the wall, mucosa congested, and on section the submucosa was red. The microscopic changes he noted were that the inner layer had almost entirely disappeared; in the submucosa there were polynuclear and mononuclear cell infiltration, new-formed vessels, and hæmorrhages; in the middle and serous layer foci of round cells were seen. In sections specially stained he found the cholera vibrio in the mucosa and deep in the submucosa.

In the cystic, hepatic, and common bile ducts, and in a lesser degree in the biliary passages in the liver, the same pathological changes were found, and a pure culture of the comma bacillus was obtained.

Table I shows the condition of the gall bladder and biliary passages as taken from cholera autopsy records of the Bureau of Science on file at the College of Medicine and Surgery, University of the Philippines.

TABLE I.—*Showing condition of the gall bladder and biliary passages.*

Year.	Cholera autopsies.	Signs of inflammation.		Stones.
		Gall bladder.	Bile ducts.	
1908	108		1	
1909	123	1		
1910	98			2
1911	1			
1912	0			
1913	79	3		
1914	226	a8		2
Total	635	12	1	4

a Thirty-nine of the gall bladders in the 1913–14 series at the time of autopsy were tied off at the common duct and sent to the Bureau of Science, Manila, where they were examined by Dr. Otto Schöbl. He records his results in a recent paper. The cholera vibrio was found in 17 of the cases on bacteriological examination of the bile. Three cases showed macroscopic pathological changes in the gall bladder, and in two hydrops cystis felleæ was found—that is, distended gall bladder containing mucus, bile of light amber color, and flaky sediment. One showed thickening of the wall with distended blood vessels, desquamation of the mucosa, and round-cell infiltration.

Table I shows that in the last two years—1913–14—there were 13 cases in 305 that showed signs of inflammation of the gall bladder, or about 4 per cent. In order to demonstrate if there were microscopic pathological changes in cases with no macroscopic lesions of the biliary passages, and to demonstrate the exact location and condition of any cases with gross or minute pathological changes, the following method was adopted. For histological examination five sections of the gall bladder and bile ducts were taken as follows:

1. Common duct.
2. Hepatic duct near the hilus.
3. Hepatic duct and liver tissue halfway between hilus and border of liver.
4. At border of liver.
5. Wall of gall bladder.

These sections were fixed in Zenker's solution in separate bottles, imbedded in paraffin in the usual manner, and cut and stained with hæmatoxylin and eosin. Twenty-eight unselected cases were examined in this manner. One of these cases (No. 3609) showed some gross pathological changes: namely, the gall bladder was dark gray and filled with thick, black bile and the blood vessels were injected. On microscopic examination the epithelial layer of the mucosa was found to be desquamated and the blood vessels of the submucosa were distended. There was

no round-cell infiltration. These changes were only in the gall bladder; the other four sections of this case showed no changes. The sections were all stained specially for the cholera vibrio with carbol fuchsin and Löffler's methylene blue, with negative results.

In the autopsies of cases in 1913–14 that were examined in the pathological laboratory of the College of Medicine and Surgery, University of the Philippines, the histological sections of the gall bladders were available for examination. By specially staining the sections for bacteria with carbol fuchsin, comma bacilli were demonstrated in two cases (autopsy Nos. 3557 and 3751). These were in all probability the cholera vibrio, but Grieg,[18] in a recent paper, cautions against error in diagnosis between the cholera vibrio and choleralike vibrio. These comma bacilli were in the mucosa and submucosa, and many resembled the involution form mentioned by Kulescha. The sections showed desquamation of the epithelial layer of the mucosa and round-cell infiltration, but no such marked changes as were recorded by Kulescha. In the cases of 1913–14 the pathological changes were not so marked as seen by Kulescha in many of his cases, but showed a catarrhal inflammation. The percentage, 4, is the same as that recorded by Grieg.

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